AN INTRODUCTION TO THERAPEUTIC INOCULATION

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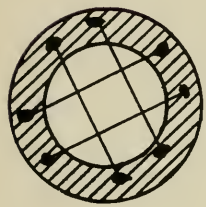
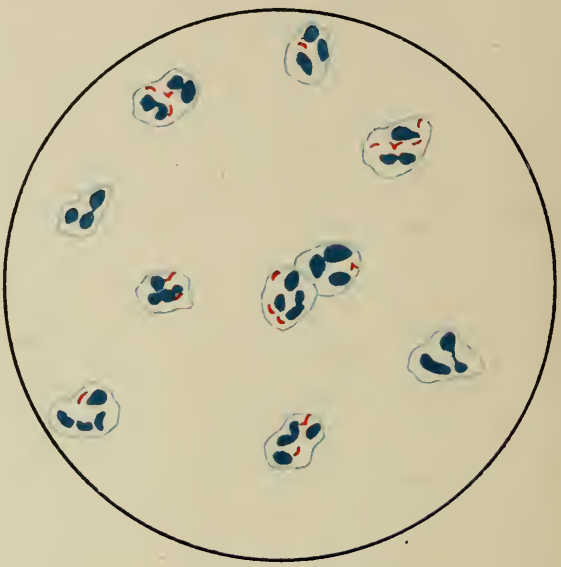
Fig. 1.

Fig. 5.





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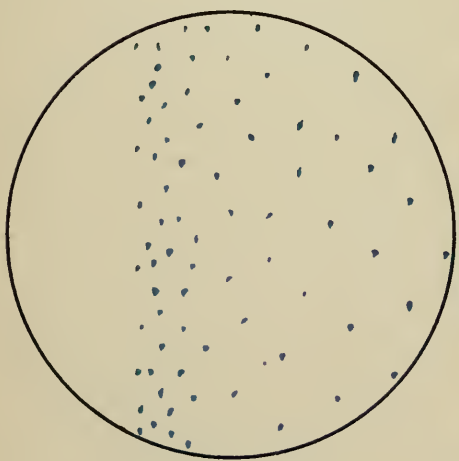

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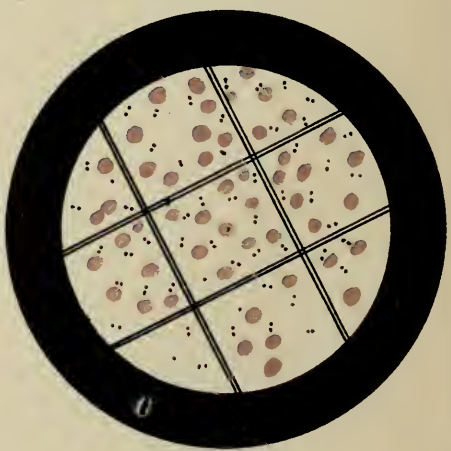

Fig. 4.

FRONTISPIECE.

FIG. A.—A slide with film spread for the estimation of the opsonic index. The drop of mixture was put down at the left-hand end of the slide, and the film spread towards the centre, consequently the white corpuscles are drawn to the extreme right-hand edge of the film.

FIG. B.—The edge of the film examined under a low power. The corpuscles can be seen collected at the edge. The white corpuscles are the only ones visible, since the red have been destroyed by the use of acetic acid. Being seen through a lens the right and left of the specimen are reversed, the edge being apparently at the left-hand extremity of the preparation.

FIG. C.—Part of the same specimen seen with a $\frac{1}{12}$th oil immersion lens. Ten leucocytes are seen in the field ; nuclei stained deep blue and their cytoplasm just distinguishable from the surrounding field, several of them have ingested tubercle bacilli, stained red. In estimating the opsonic index, it is required to count 100 such leucocytes in each specimen with their contained bacteria. The outlines of a few red corpuscles are faintly visible. Specimens A, B, and C are stained with carbo-fuchsin and methylene blue.

FIG. D.—The method of estimating the strength of a vaccine. The suspension of bacteria is mixed with normal blood in known proportions and spread on to a film. The bacteria are seen lying among the red corpuscles. The square is made by a network of fine glass filaments in the eyepiece of the microscope to improve facility in counting. The numbers of corpuscles and bacteria are estimated in a series of fields. Specimen stained with carbol-thionine.

FIG. E.—Cardboard disc to be dropped into the eyepiece showing the method of fixing the glass filaments. Actual size.

An Introduction to Therapeutic Inoculation

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MACMILLAN AND CO., LIMITED
ST. MARTIN'S STREET LONDON

1911

Richard Clay and Sons, Limited,
bread street hill, e.c., and
bungay, suffolk.

PREFACE

This book is divided into two parts under the headings of "Principles" and "Practice." The first part is an attempt to state in the simplest possible terms the common sense of Therapeutic Inoculation, the object of which is to induce immunity to bacterial infections. Immunity is a subject which is extremely complex in its details, but some of its main facts are quite simple, and it is only these facts of which we are at present in a position to take advantage in the treatment of disease, and on them the principles of Therapeutic Inoculation are founded. Only such experiments are quoted as illustrate these principles, and all advanced theoretical work, such as the side-chain theory, is omitted. The principles stated, however, are believed to be of the very first importance, and it is hoped that some iteration will therefore be pardoned.

The second part deals with their practical application, and some technical details which are described in the first part for the sake of clearness have been repeated in the second for the sake of completeness, and to avoid cross-reference.

This part of the book deals with the treatment of some infections by means of therapeutic inoculation. Only those infections of which there has been large experience are considered, and isolated or exceptional cases are rarely mentioned.

Under each disease the number of cases treated is given, with the results and the doses of vaccine administered, so that opportunity is afforded for consideration of the grounds on which the treatment is recommended.

The principal sources of information have been the Out-patient records of the Department of Therapeutic Inoculation at St. Mary's Hospital, together with papers published by individual workers in the Department, and records of cases treated by the writer. The Out-patient records, some 1500 in number, are, as is necessarily the case, very incomplete, and there are comparatively few of the total number of cases which are sufficiently fully described for correct inference to be drawn from them. However, between six and seven hundred have been used. No selection of cases has been made, all results, good, bad, and indifferent, which have been sufficiently clearly indicated in the notes, have been recorded, and every effort has been made to allow an unbiassed estimate of the value of this method of treatment to be formed. The published papers to which reference is made are those of Mr. Alexander Fleming, F.R.C.S., on acne, Drs. Ross and Johnson on erysipelas, Dr. Freeman on whooping cough, Drs. Willcox and Parry Morgan on pneumonia, and Mr. Maynard Smith, F.R.C.S., on tubercular arthritis, and use has been made of an unpublished communication by Dr. John Matthews on mucous colitis. To all these gentlemen the writer wishes to express his indebtedness.

The writer has thus used cases which he has either seen personally, or which have been treated under conditions with which he is familiar, or about which he has been able to obtain first-hand information. The cases of which any individual can have even this amount of personal experience are necessarily limited in number and unequal in distribution, but since a considerable number have been accessible it has been thought best only to make use of records of cases

treated under a uniform system. The writer accepts the responsibility for all opinions expressed.

The technique described is substantially that employed in the Laboratory at St. Mary's Hospital. A large number of workers are employed there and small variations in method are used by different individuals. The actual measurements given in the text are those used by the writer; they were learnt at St. Mary's during a period of nearly four years, and some detail or other was picked up from nearly every member of the staff; other details again are merely personal habit. To make full acknowledgement to all who have taught him would not be possible. Some further acknowledgements must, however, be made. To Sir Almroth Wright, M.D., F.R.S., the Director of the Department of Therapeutic Inoculation at St. Mary's Hospital, to whose intellectual power, technical ingenuity and personal enthusiasm the present position of this method is almost wholly due, the writer owes his most grateful thanks for his generous permission to use the Laboratory and the records of the Department, and for much illuminating suggestion and criticism; without his sanction this book could not have been attempted. His thanks are due also to Dr. F. C. Martley for much critical help; to the Editor and Publisher of *Science Progress*, and the Editor of the *British Medical Journal* for permission to use parts of articles published in their periodicals; to Drs. Bulloch and Macdonald and to Dr. Giglioli of Florence for the use of charts which are indicated in the text; to Mr. Stewart Bosworth, B.Sc., for the illustrations, and to Miss May Hewlett for clerical assistance. Finally he must express his appreciation of the consideration he has received from the Publishers, and their ready acquiescence in all suggestions.

WIMPOLE STREET, W., 1911.

CONTENTS

PART I.—PRINCIPLES

PART II.—PRACTICE

CHAPTER VIII

CHAPTER IX

CHAPTER X

CHAPTER XI

CHAPTER XII

APPENDIX

LIST OF ILLUSTRATIONS

PLATES (*at end*)

PART I

THE PRINCIPLES OF THERAPEUTIC INOCULATION

AN INTRODUCTION TO THERAPEUTIC INOCULATION

PART I.—PRINCIPLES

CHAPTER I

ON SPONTANEOUS RECOVERY FROM BACTERIAL INFECTIONS

It is well known that numberless diseases are the result of infection with pathogenic bacteria. The first essential for success in the treatment of any disease is the removal of the cause, hence for the successful treatment of bacterial disease it is necessary to remove the infecting organisms. Therapeutic Inoculation is a method of treatment of bacterial disease which is directed solely to this end.

When bacteria are introduced into the body, certain physiological reactions ensue with the production of substances which are destructive of the organisms, and therapeutic inoculation is based upon the study of those reactions. Some consideration of the processes involved is therefore necessary, before discussing the method by which these natural powers can be stimulated by artificial means in the treatment of disease.

In the present chapter some account is given of the phenomenon of spontaneous recovery, and of the causes upon which it depends.

When a bacterial infection occurs and is left untreated, it terminates in one of three ways, in death, in recovery, or in chronicity.

Both the two former results are familiar in the case of pneumococcus pneumonia, which is, bacteriologically speaking, an infection of the lungs with the *micrococcus lanceolatus* of Fränkel. Such results show, first, that the human body has the power of getting the better of bacterial infections, and secondly, that this power frequently breaks down. But, as has been said, all infections do not end in death or recovery; a condition of "chronicity" often results, in which case there is a permanent infection, generally localised and not of great severity, which persists indefinitely and grows gradually worse. This condition may arise if the pneumococci infect a joint instead of a lung, and give rise to chronic pneumococcal arthritis. In this case the resistance of the body has been sufficient to prevent death, but insufficient for recovery, and the machinery of resistance may be considered to have partially broken down.

The aim of therapeutic inoculation is to stimulate the natural powers of recovery by means which are specific for each separate infection. Its most valuable application is in stimulating those powers in the chronic cases in which the natural mechanism has partially failed. To understand the principles on which it is applied it is necessary to consider what occurs in cases of spontaneous recovery, since therapeutic inoculation makes use of the mechanism involved in that process.

The phenomenon of spontaneous recovery from bacterial infections is a very remarkable one, as is readily seen on consideration of the sequence of events in an acute infection, such as pneumonia. This is what frequently occurs : a man in good health becomes infected with pneumococci, probably in no very great number. They multiply, and become infinitely numerous. The man develops pneumonia,

and in a few hours is brought to the point of death. After a few days of severe illness the *crisis* occurs, and the patient

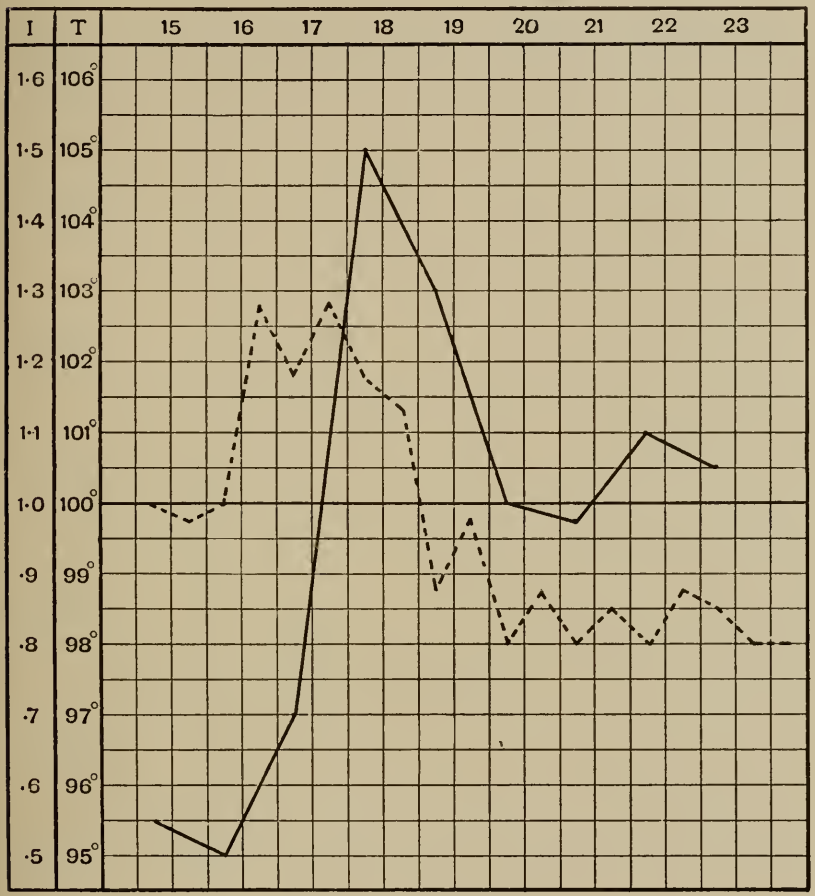


CHART I.—PNEUMONIA

Pneumonia undergoing rapid resolution. ———— Curve of antibodies. (I.) ---------- Curve of temperature. (T.) Slightly modified from "Immunity in Pneumococcal Infections" by G. G. MacDonald in "Studies in Pathology." Edited by W. Bulloch.

recovers, and his tissues become free of pneumococci; though his sputum may still contain them.

How is this paradox to be explained? The man's body

when infected in health could not prevent a few organisms multiplying into many, and yet, when weakened by disease, it has ultimately rid itself of countless millions. The

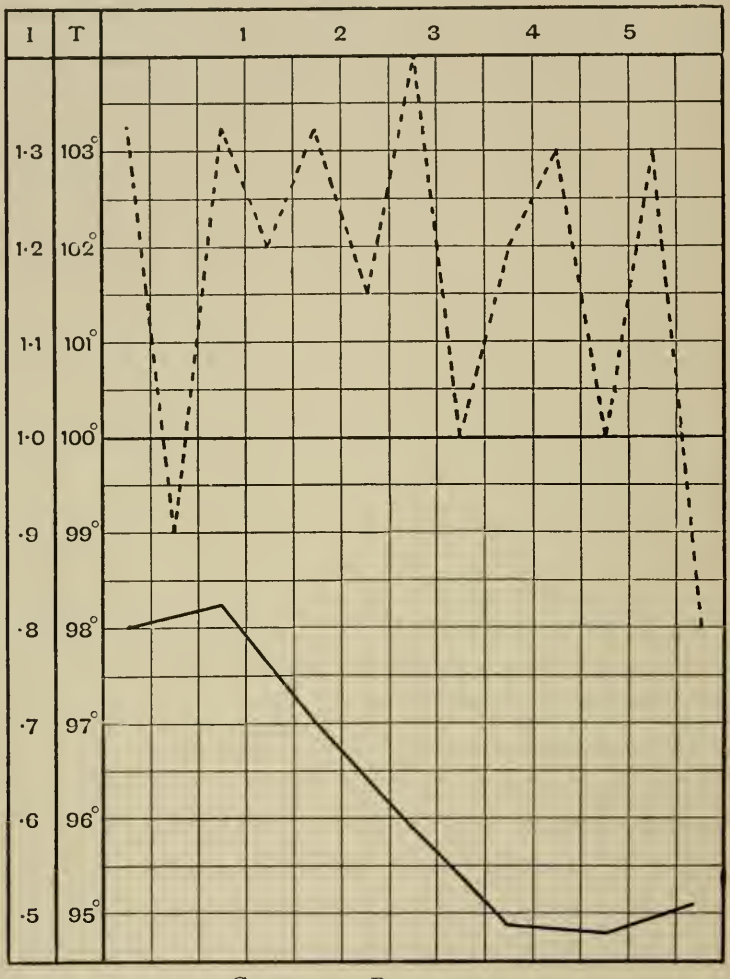


CHART 2.—PNEUMONIA

Pneumonia ending in death. (MacDonald, *op. cit.*). ——— Curve of antibodies. (I.) ---------- Curve of temperature. (T.) Slightly modified from the original.

reason for this is that the man's tissues have elaborated *antibodies*, or substances which are antagonistic to the invading pneumococci.

The formation of antibodies is the essential process upon which all natural recovery depends, and constitutes the rational basis of all therapeutic inoculation. Some account of this physiological property of the animal body is therefore required.

Repeated experiments have shown that if a foreign albuminous substance, which is soluble in the body fluids, is introduced into the tissues of a living animal, it stimulates the tissue-cells to form *antibodies* which neutralise or destroy it. One classical experiment may be quoted in illustration. If the red blood-corpuscles of an animal of one species, for instance, a sheep, are incubated *in vitro* with the serum of an animal of another species, for instance, a rabbit, the sheep's corpuscles remain almost intact, and fall down as a red deposit at the bottom of a tube of nearly colourless serum. But if a rabbit is inoculated with sheep's red corpuscles, the rabbit's tissues develop antibodies to these foreign albuminous substances, and if the serum of a rabbit so inoculated is now incubated with fresh sheep's corpuscles, the latter are "hæmolysed," that is, the corpuscles are destroyed, the hæmoglobin escapes, and a clear red solution of hæmoglobin is produced in the serum, in striking contrast to the nearly colourless serum of the previous experiment. It is important to note that though the serum of a rabbit inoculated with sheep's corpuscles has the power of hæmolysing sheep's corpuscles, it produces no effect on those of an animal of another species: for example, the corpuscles of a dog or an ox will be unaffected by it. That is, the antibodies formed are *specific*.

After suitable inoculation the rabbit's serum has acquired the power of hæmolysing sheep's corpuscles. Not only have the rabbit's tissues produced antibodies which have destroyed the sheep's corpuscles introduced into them, but also a residue of antibody, over and above the amount required

for that purpose, has been produced, which has passed into the rabbit's serum, and is available against fresh corpuscles, as has been demonstrated *in vitro*. That is, antibodies are produced *in excess* of the amount required to neutralise the quantity of foreign substance introduced into the tissues.

If, instead of sheep's corpuscles, any of the following albuminous substances :—ricin [1] (a vegetable hæmagglutinative poison), spermatozoa (a suspension of animal cells), or milk (an animal secretion)—are injected into animals, antibodies are formed in excess, which pass into the serum and can be demonstrated by suitable experiments. The serum will destroy the hæmagglutinative power of ricin, or inhibit the movements of spermatozoa, or coagulate milk, as the case may be. These experiments show how catholic is the power of the tissues to elaborate antibodies to foreign albuminous substances.

The chemical composition of antibodies is unknown, none of them have been crystallised or otherwise procured in pure form; all that is really known is that "lytic," or similar reactions can be demonstrated with the serum of suitably prepared animals, but for simplicity's sake, and to avoid circumlocution, it is usual to assume that these reactions are due to separate substances and to speak of them as specific antibodies. The foreign substances stimulate the tissues to form antibodies to them, in accordance with the general law that *the animal organism elaborates antibodies to any foreign albuminous substance introduced into it, which is soluble in the tissue fluids, and does so in excess of the amount required to neutralise the quantity of foreign substance introduced.*

[1] Ricin is a substance derived from castor-oil seeds. When it is added to red blood-corpuscles they are agglutinated and destroyed. If an animal is inoculated with suitable doses of ricin, its serum develops antibodies to ricin, and if ricin is incubated with this serum, it loses its power of destroying red corpuscles.

Bacteria are among the substances which stimulate the tissues to form antibodies in excess, and these antibodies can be demonstrated by suitable experiments in the serum of inoculated animals. They can be readily demonstrated in human serum after inoculation, and since the present volume deals with the treatment of human disease, the reactions of human tissues and sera are those considered in the following pages.

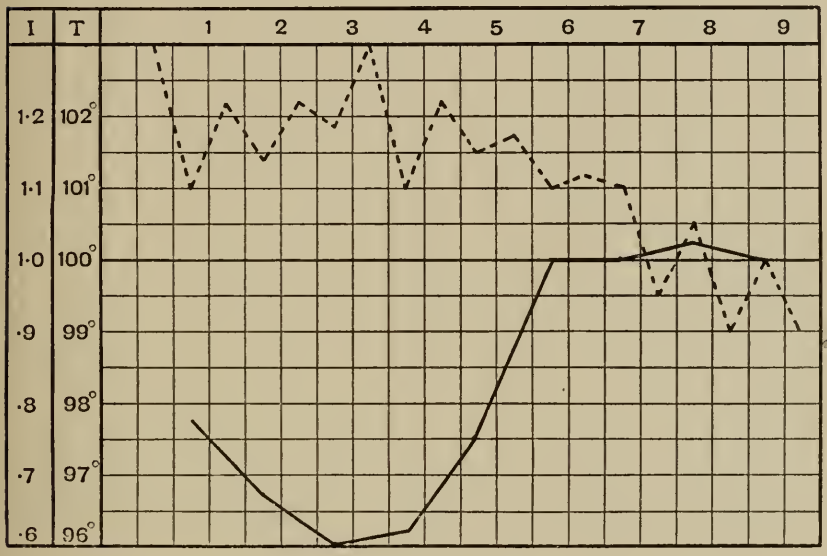


CHART 3.—PNEUMONIA

Pneumonia undergoing gradual resolution. (MacDonald, *op. cit.*) Slightly modified from the original. ———— Curve of antibodies. (I.) ---------- Curve of temperature. (T.)

Antibodies to bacteria differ from those previously described in one respect, though the difference is one of degree rather than of kind. As has been stated, the serum of a rabbit has practically no hæmolytic power on the corpuscles of a sheep, unless it has been inoculated with them, but the serum of a healthy man normally contains antibodies to the pathogenic bacteria. Healthy human serum produces *in vitro* a definite and measurable effect on most,

at any rate, of the micro-organisms causing disease, therefore, for simplicity's sake, normal serum is said to contain certain *antibacterial substances*, and for the present it may

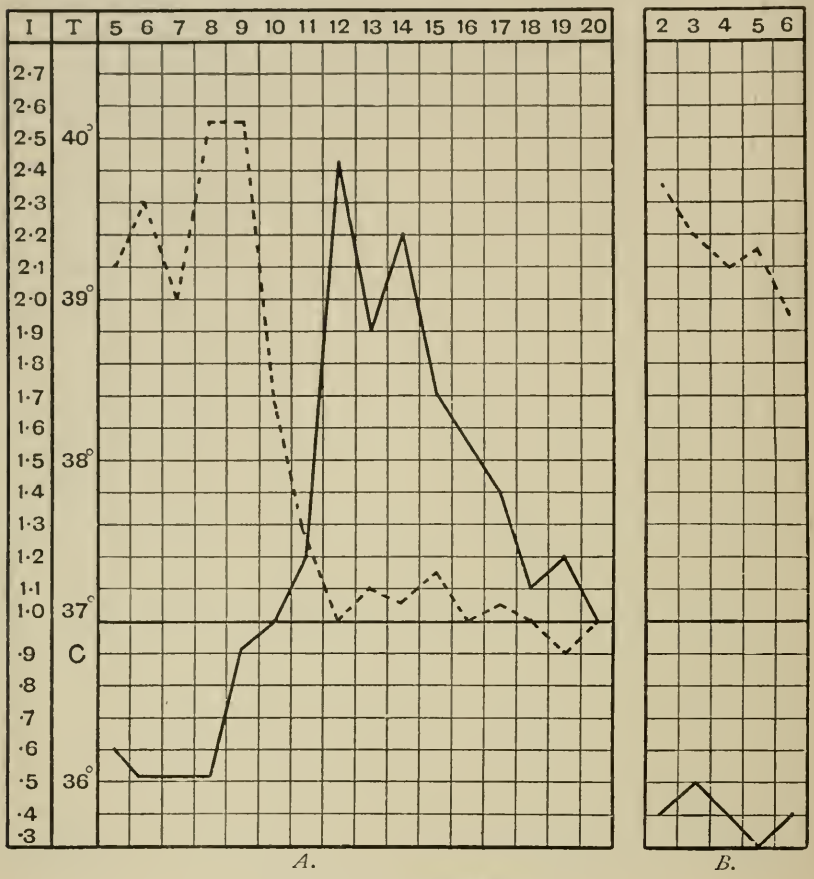


CHART 4.—PNEUMONIA

A. Recovery. B. Death. ———— Curve of antibodies. (I.) ---------- Curve of temperature. (T.) From "Interno alle Modificazione dell' indice opsonico nel corso di alcune malattie acute da infezione." (Giglioli & Stradotti.)

be assumed that all normal sera contain them in approximately equal amounts.

If a man is inoculated with bacteria in suitable doses, his tissues are stimulated to increase the normal quantity of

antibodies, in excess of the amount required to counteract the stimulus, and this increase can be demonstrated in his serum. That is, the result of a suitable inoculation with bacteria is apparent rather as an increase in the amount of a substance normally present than in the development of a new substance.

The antibacterial properties of serum, and their increase after inoculation, are well illustrated by the action of human serum on the *bacillus typhosus*. If a volume of a broth culture of typhoid bacilli which contains ten million organisms per cubic centimetre is incubated with an equal volume of normal serum, the bacilli will all be killed, and if the mixture is subsequently planted out on agar no growth will result.[1] If the culture is appreciably stronger than this, containing, say, twenty million bacilli, the antibacterial substances will be insufficient to kill all the bacteria, and when the mixture is planted out, some colonies will grow. But if the serum of a person who has been successfully inoculated with typhoid bacilli is employed, an equal volume of a broth culture containing one hundred million bacilli per cubic centimetre will be rendered sterile. This is proof that the tissues have been stimulated to an increased formation of antibacterial substances, and that the latter have been increased tenfold.

This phenomenon, which is known as the "bactericidal reaction," is very clear evidence of an increase in antibody-formation, but it is one which can be demonstrated *in vitro* with only a few varieties of bacteria; with staphylococci for instance, no such reaction can be shown. There

[1] In practice a broth culture of typhoid bacilli, of twelve hours' growth, is taken, progressive dilutions of 1 in 10, 1 in 100 and so on are made, and a series of bactericidal experiments are made with the different strengths, only very small amounts being actually used. It is estimated, however, that the volume of culture which is sterilised by the addition of an equal volume of normal serum contains ten million bacilli per c.c.

are, however, other antibacterial reactions, which are increased after inoculation and which can be demonstrated against nearly all organisms, though by less direct methods.

Only the antibodies to the particular organism injected are increased, just as the injection of the red blood-corpuscles of an animal of any species leads to the formation of antibodies to the corpuscles of animals of that species only. The inoculation of pneumococci will not

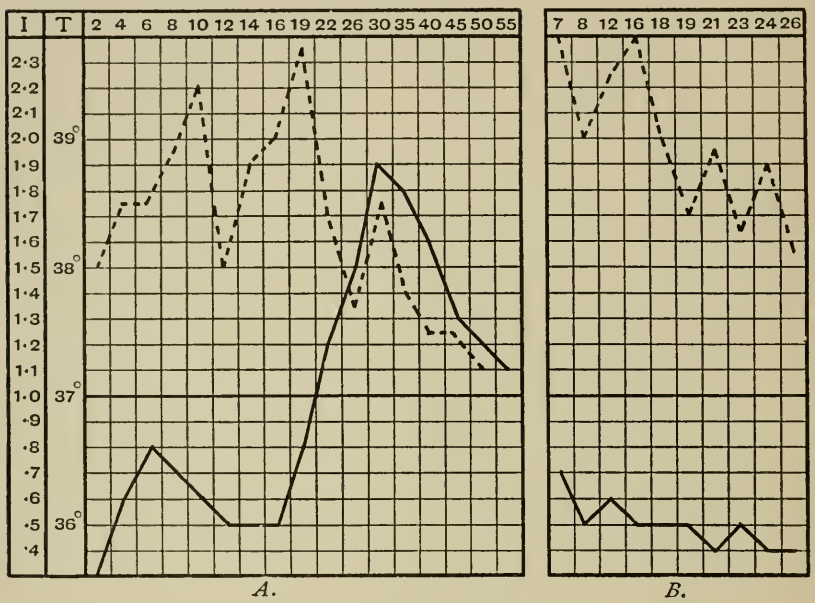


CHART 5.—MENINGITIS

A. Recovery. B. Death. ———— Curve of antibodies. (I.) ---------- Curve of temperature. (T.) (Giglioli & Stradotti, *op. cit.*)

lead to an increase in antitubercular substances, which will be quite unaffected, while the antipneumococcal substances will be increased. That is, the antibacterial substances formed are specific.

The formation of antibacterial substances as a result of the experimental introduction of bacteria into the tissues is, therefore, demonstrable, and there is clear evidence that

animal tissues form antibodies to foreign albuminous substances in general, and to bacteria in particular.

But infective disease results from the involuntary introduction of bacteria into the tissues. Pneumonia, to return

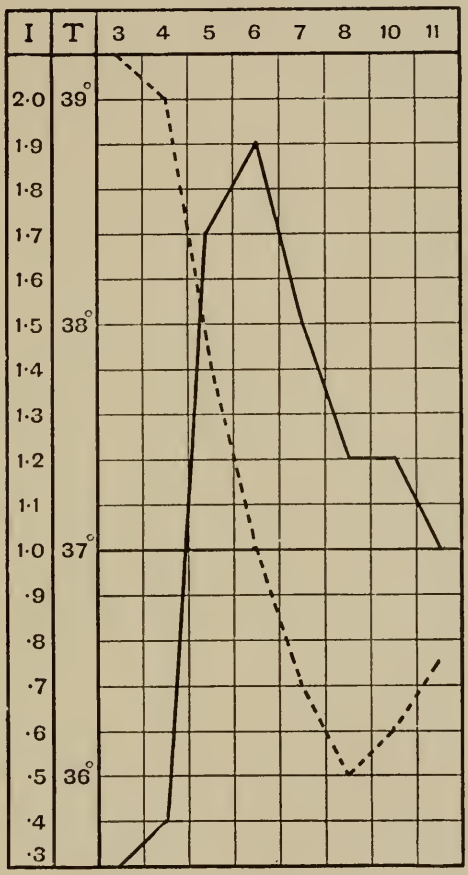


CHART 6.—ERYSIPELAS

Recovery. (Giglioli & Stradotti.) ——————— Curve of antibodies. (I.) ---------- Curve of temperature. (T.)

to the instance first considered, is due to the introduction of pneumococci into the tissues of the lung. Is there evidence that this leads to the formation of antibodies to the pneumococcus?

If the blood of a person suffering from pneumonia is examined and compared with that of a normal person, it is found that during the early stage the patient's serum contains a smaller amount of antibodies to pneumococcus than that of a normal person. This may be expressed

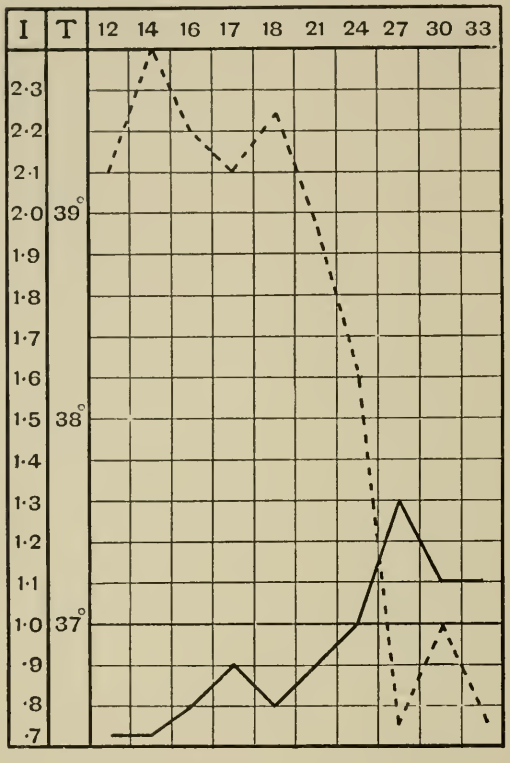


CHART 7.—TYPHOID FEVER

Recovery. (Giglioli & Stradotti.) ———— Curve of antibodies. (I.) ---------- Curve of temperature. (T.)

more shortly by saying that the patient's *resistance* to the pneumococcus is subnormal. But at the period of *crisis* there is a large and sudden increase in the amount of these bodies to an extent far exceeding the normal. In cases which end by *lysis* a similar but smaller rise occurs, and in cases which end fatally, there is no rise at all. These

points are illustrated in Charts 1, 2, 3, and 4, which are taken from actual cases of pneumonia.

The same reaction may be demonstrated in many other infections besides pneumonia. Chart 5*A*. illustrates a similar rise in antibodies to meningococcus, in a case of cerebrospinal meningitis ending in recovery, and Chart 5*B*. shows the absence of such a rise in a fatal case of the same infection. Charts 6 and 7 show a rise in antibodies to the

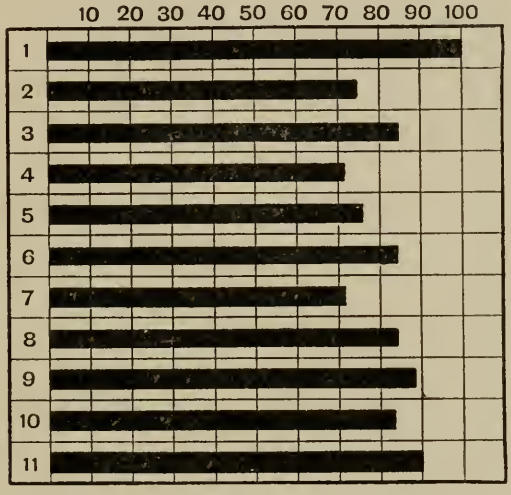


CHART 8

Percentages of antibodies to the *bacillus tuberculosis* in the serum of a series of cases of chronic tubercular infection, after rest. No. 1.—Normal serum, 100 per cent. Nos. 2–11.—Patients, amount of antibodies subnormal in all cases. (Compiled from the records of the Department of Therapeutic Inoculation, St. Mary's Hospital.)

infecting organisms in the course of recovery from erysipelas and typhoid fever.

Since the tissues have the power of forming antibodies to foreign albuminous substances in general, and bacteria in particular, and since spontaneous recovery from bacterial infections is coincident with excessive production of antibodies to the infecting organism, while in fatal cases the

antibodies present in the serum are much below the normal, it is concluded that spontaneous recovery is due to the formation of antibodies to the infecting bacteria.

But failure to recover from bacterial infections does not always end in immediate death; chronic infection is a far commoner result. Chart 8 shows the percentage of antibodies, in the serum of ten cases of tubercular infection, who had been kept at rest before examination, compared with the amount present in normal serum. It will be seen that the antibodies are distinctly subnormal in quantity. It is fair to infer that chronicity is due to an imperfect response to the stimulus of the bacteria on the part of the tissues.

These facts may be summarised as follows:—It is a physiological property of animal cells to form antibodies in excess to any foreign albuminous substance introduced into the tissues, which is soluble in the tissue fluids. Spontaneous recovery from pneumonia, or any other infective disease, is due to the adequate formation of antibacterial substances by the tissues; a total failure in the formation of antibodies ends in death, while an inadequate formation leads to a condition of chronic infection.

It is well known that a person who has recovered from an acute bacterial infection is not liable to contract the same disease again for a longer or shorter period. Such a person is said to be "immune" to such and such a disease. It is probable that such post-infective immunity is due to the production of antibodies in excess as the result of the presence of bacteria in the tissues, and it is assumed that such a person remains immune for so long as the excess of antibodies is present in his blood.

The patient's tissue-cells have made the physiological effort of producing these bodies, and in consequence such immunity is spoken of as "active" immunity.

We therefore proceed on the assumption that natural recovery from infective disease, and the resulting immunity, depend upon the successful formation of an excess of antibodies by the tissues as a result of the stimulus supplied by infecting bacteria.

CHAPTER II

ON THE ARTIFICIAL STIMULATION OF THE NATURAL POWERS OF RECOVERY

INFECTIVE disease is due to the accidental introduction of pathogenic bacteria into the body. Natural recovery and active immunity result from the formation by the tissues and presence in the blood of specific antibodies to the invading bacteria.

It is obvious that the same antibodies and the same immunity will be produced if the bacteria are experimentally introduced into the tissues. But the introduction of living bacteria leads to infection with disease. If, however, these bacteria are killed by heat, their protoplasm retains its specific character, and if bacteria so killed are injected into the tissues, antibodies are formed, and immunity is acquired without any risk of infection with the disease.

This method of inoculating with dead bacteria with a view to producing active immunity is in fairly common use as a prophylactic measure against typhoid fever, and to a less degree against cholera and plague. A pure culture of the infecting organisms is obtained, and suspended in normal salt solution; the suspension is sterilised by heat, and standardised, that is, the number of bacteria per unit

volume is ascertained. This suspension is called a *vaccine*, and in this book the term *vaccine* indicates a sterilised suspension of bacteria of known numerical strength.

The prophylactic use of vaccines, the attempt to supply a person with a given set of antibodies before exposure to infection, is reasonable enough. When a person anticipates exposure to infection with typhoid fever, he undergoes prophylactic inoculation, so that if any typhoid bacilli gain entrance to his tissues they will be there met by tissue fluids rich in antityphoid substances, and the bacilli will consequently be destroyed, and the individual will escape.

The employment of prophylactic inoculation against all bacterial infections is not practicable on account of the very variable persistence of acquired immunity. For instance, a person who has recovered from variola is protected for life, while in other diseases, such as influenza, the immunity acquired in recovery is very transient, and in the same way, after prophylactic inoculation, the immune period varies with the organism used, and for reasons which are still unknown. Typhoid fever, cholera, and plague are the only diseases against which prophylactic inoculation is used to any extent at the present time.

The use of vaccines is not, however, limited to prophylaxis; they have a far wider application in the curative treatment of bacterial disease. The advance from prophylactic to curative inoculation is clearly a great step, and it is one which had to be taken against considerable opposition. The obvious criticism of such treatment is that it is absurd to introduce more bacteria, dead or alive, into the tissues of a patient who is already suffering from more than he can deal with. To meet this criticism it is necessary to follow the supposed course of events when bacteria enter the body and cause disease. What follows is not based on precise experimental evidence, but is offered as a

working hypothesis which gives a reasonable explanation of the facts.

Bacterial infections may be general or local. General infection or septicæmia results when bacteria enter the blood and multiply there. But on entering the blood stream, they are exposed to the antibacterial substances there present, and are generally killed, hence primary septicæmia is rare in comparison to local infection. But on entering the blood a few bacteria may escape death and become lodged among the tissues, and set up local infection, or the tissues may be invaded directly without passage through the blood stream; thus the lymphatic glands may be infected from the tonsil, or the skin through a hair follicle, or the lung-tissues may be invaded by inhaled pneumococci. When bacteria thus become lodged in the tissues it appears that in some way they have avoided, or have been able to resist, the protective substances of the blood. Inflammatory reaction occurs around them, and they are in this way partially cut off from the lymph stream. Lymph is collected at the site of infection, and the stream, though not completely stopped, is slowed; the importance of local treatment of this condition is referred to in a later chapter. The position is now that of a struggle between the bacteria, producing toxins, and the tissue-cells, producing antibodies. When the advantage is on the side of the bacteria, the cells are killed, and the bacteria are then free to multiply among dead cells which are incapable of forming antibodies. This is the state of affairs in early stages of an acute local infection. If a person suffering from a lesion of this kind is kept strictly at rest and his blood is examined for antibodies to the infecting organism, they are generally found to be subnormal. It is believed that the antibodies have been exhausted in an unsuccessful attempt to destroy the bacteria which consequently have gained foothold. If the lesion is so situated that fluid, such as the pus from an abscess or effusion from

an inflamed serous or synovial cavity, can be removed from it for examination, the fluid will be found to be still poorer in antibodies than the circulating blood.

When recovery occurs it is supposed either that the focus of bacteria grows sufficiently to allow of organisms coming into contact with healthy cells which form the requisite antibodies, or, as is perhaps more probable, migratory cells are attracted to the infected area, or possibly bacteria escape from the focus into the blood stream and lodge elsewhere among healthy cells. In any case the bacteria come into contact with healthy cells, antibodies are formed in excess of the amount required to destroy the organisms which afford the stimulus, so that the residue passes into the blood and lymph and helps to destroy the bacteria still in the lesion. This phenomenon is called *auto-inoculation.*

Chronic infection is a state in which efficient auto-inoculation does not occur. In a case of chronic infection in which this is the case, the cells at the site of infection are exhausted and the healthy tissues are not called into play. If now a vaccine is introduced into a site which is free of infection, the tissues at this point will be stimulated to produce antibodies in excess of the amount required to counteract the vaccine, and the excess will pass into the blood stream, and so into the lymph, and will be available against the organisms at the site of infection, as in the previous case.

Chronic infections are those which are best adapted for treatment by means of vaccine therapy.

It will be of assistance to consider the application of this method of treatment in a concrete instance, which will also draw attention to certain precautions which it is necessary to observe in its use, and the reasons for them.

If a patient suffering from chronic tubercular arthritis is

kept strictly at rest for a period of forty-eight hours, care being taken to avoid any irritation or active or passive movement of the affected joint, it will be found that his blood is subnormal in antitubercular substances (*see* Chart 8). If now a suitable dose of tubercle vaccine, containing about 1/15,000 of a milligramme of sterile comminuted tubercle bacilli, is injected into a healthy site, and the patient's tissues respond well to this stimulus, fresh antitubercular substances will be formed in the course of a few hours; the blood will become richer in those substances (it may approach and even exceed the normal in this particular), and there may already be some subjective improvement in the patient's condition.

But if, instead of the small dose of 1/15,000 milligramme, a dose of 1/2000 milligramme is given, the results will be quite differerent; at first the antibodies in the patient's blood will not be increased, but will be reduced still lower, and the clinical condition will change for the worse. After a few days a rise in antibodies and clinical improvement may supervene. The reasons for this condition appear to be as follows. In the vaccine a large mass of foreign albuminous substance has been introduced into the tissues, the antibodies present in the blood are fixed thereby, and even less than usual are available against those in the lesion, and some time elapses before the tissues are able to respond to the stimulus and form fresh antibodies; meantime the bacteria in the lesion are without the little restraint which is commonly opposed to them, and are able to aggravate the condition. *See* Chart 9.

This period of depression of antibodies is known as the "negative phase," and if during this phase a further inoculation is given, the phase will be increased in length and severity. It is essential to recognise that the doses of vaccine which satisfactorily stimulate an immunising response are strictly limited in size: it is by no means true

that the larger the dose, the greater is the formation of antibodies.

The case under consideration is one of arthritis which is supposed to have been kept strictly at rest, and it is supposed also that the patient's blood while at rest is poor in antibodies. If the affected joint is exercised by active muscular work, or massaged, or congested by bandages applied over its efferent veins, or in any way subjected to

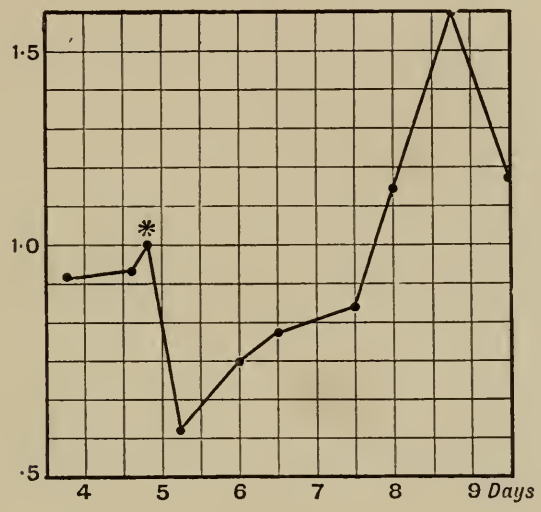


CHART 9

Case of Tuberculosis. Curve of antibodies. Production of "Negative Phase." At * a dose of Tuberculin was injected: observe the immediate fall in the amount of antibodies. (From the Records of the Department of Therapeutic Inoculation, St. Mary's Hospital.)

hyperæmia, the blood-supply to the part will be increased in force or volume, and bacteria will be washed out of the lesion and will lodge among other tissues. By this means an auto-inoculation is induced, and according to its magnitude it will be found that the antibodies in the blood have been either depressed or increased.

It is clear, therefore, that a dose of vaccine which is of suitable strength for a resting patient may become much

too large in the presence of auto-inoculation, and it is essential to the success of vaccine therapy to keep auto-inoculation under control. This is the reason why localised infections are those best adapted for vaccine treatment. In septicæmia, where organisms are multiplying in the blood, it is manifestly impossible to control the auto-inoculations, consequently such conditions are not very suitable for this method; however, when organisms are multiplying in the blood, they are not necessarily lodging in the tissues which subserve the function of forming antibodies, and by the use of minimal doses of vaccine, which are themselves

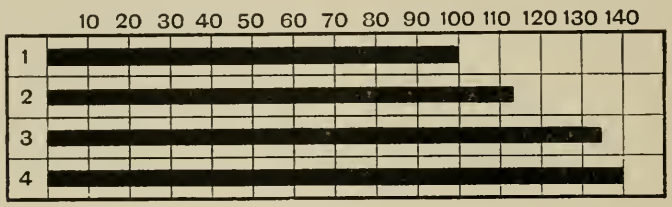


CHART 10

Percentage of antibodies to gonococcus in cases of gonococcal arthritis kept at rest. 1.—Normal serum, 100 per cent. 2, 3, 4.—Patients' serum, showing percentages above normal in chronic infections. (Compiled from the Records of the Department of Therapeutic Inoculation, St. Mary's Hospital.)

insufficient to induce a negative phase of any magnitude, many cases have now been successfully treated.

At this point it is necessary to make a short digression in the interests of strict veracity. The suggestion was made in the first chapter that in chronic infections the serum is subnormal in antibodies to the invading bacteria, and several instances of this condition in tubercular disease have been cited. That is the usual condition and the one which makes the rationale of this method most easy to understand. It is, however, conceivable that in chronic disease the tissues may respond to the stimulus of the bacteria and the serum may contain more antibodies than

normal; but this amount may be insufficient to get rid of the infection, a great excess being sometimes required for this purpose, so that in chronic disease the mere presence of more than the normal amount of antibodies is no proof of a condition of recovery. This is often the case in

CHART II

Part of a chart from a case of Glanders which ended fatally. Curve of antibodies, showing abnormally high readings in a chronic infection. (From the Records of the Department of Therapeutic Inoculation, St. Mary's Hospital.)

gonococcal infections, as is illustrated in Chart 10. Chart 11 illustrates excess of antibodies in a chronic case of human glanders which was not improving clinically, and which ultimately ended fatally. These are, however, exceptions; in most cases of chronic infection the antibodies are subnormal when the patients are kept at rest.

The following, in brief, is the aim of those who employ vaccine therapy. Recovery and active immunity are the result of the formation of antibodies consequent on the presence of foreign albuminous substances in the tissues. Dead bacteria stimulate this formation no less efficiently than living ones, and without risk of infection. Vaccines made of dead bacteria may therefore be injected into healthy persons as a prophylactic measure to provide them with a given set of antibodies in case of exposure to a particular infection, or they may be injected into healthy sites in the persons of patients suffering from localised infection to stimulate the formation of antibodies when the natural mechanism in the neighbourhood of the lesion has broken down. It is important to bear in mind that a vaccine is not an antidote to an infection, but a stimulus to the body to form its own antidotes.

Serum Therapy.—If the injection of a vaccine into a patient leads to the appearance of specific antibacterial substances in his serum, the question arises if it is practicable to employ the serum of an immunised animal in the treatment of an infection. The idea is an attractive one. It appears possible by this means to flood the body of a patient with a serum rich in ready-made antibodies, which are available against his infection, without any physiological effort on the part of his tissues in elaborating them. Vicarious protection of this kind is called "passive" immunity. In practice, however, this promise is fulfilled only in some special instances.

Bacterial infections are of two kinds: in the first kind there is a single focus containing relatively few organisms, each of which produces a large amount of toxin; in the second kind there are relatively many organisms each of which produces a minute amount of toxins.

The best known examples of the first kind of infection

are diphtheria and tetanus. In these diseases the patients suffer from poisoning by bacterial products, or toxæmia. When these bacteria are cultivated artificially the toxins are produced, and can be obtained apart from the bodies of the organisms, and are therefore known as "exotoxins." If these exotoxins, which are albuminous substances, are injected into animals, *antitoxins* appear in the serum, which have the power of neutralising toxin in a manner comparable to the neutralisation of an acid by an alkali, though the reaction differs from a simple chemical one. The correct treatment of bacterial toxæmias is by means of antitoxic sera. Definite units of toxin and antitoxin have been agreed upon, and the antitoxic sera for diphtheria and tetanus are accurately standardised. Their successful use depends upon the fact that horses, which are the animals used for the preparation of antitoxic serum, have very remarkable powers of responding to inoculation with toxin, and produce an enormous excess of antitoxin. The animals are injected with increasing doses of toxin over a long period of time, and their tissues do not lose their power of responding to the stimulus, but elaborate more and more antitoxin. The result is that sera are obtainable which really are highly concentrated solutions of antitoxin, and enough antitoxin can be injected in small bulk to neutralise all the toxin in the patient's blood; and it appears that in the treatment of these toxæmias, neutralisation of the toxin is all that is required, the tissue-cells being presumably sufficient to deal with the bacteria in the absence of toxin.

In the second kind of bacterial infection each organism produces a minute amount of toxin, called "endotoxin" because it cannot be obtained from cultures apart from the bacterial bodies, so that an antitoxic serum cannot be produced. The toxins are intimately bound up with the bacterial protoplasm, and although by comminution and

various physical processes it is said to be possible to isolate endotoxins, in practice it is necessary to attack the bacteria themselves. Many attempts have been made to produce bactericidal sera, but on the whole with disappointing results. It is claimed, however, that sera of considerable prophylactic value can be produced against typhoid, cholera, dysentery and the streptococcal infections, and of slight value against some others. They have no curative value. The sera are tested by the degree of immunity conferred on animals by the serum against otherwise fatal doses of the organisms.

There are certain theoretical objections to the use of bactericidal sera. It has been explained that only limited doses of vaccine act as immunising stimuli; excessive doses depress rather than stimulate the production of antibodies. As the result of a successful prophylactic inoculation for typhoid the bactericidal substances in the blood are increased about ten-fold, but this is evidence of much greater increase than can be demonstrated with most organisms, so that the antibacterial sera cannot be very rich in antibacterial substance, whereas an antitoxic serum is extremely rich in antitoxin. Ten cubic centimetres of serum is as much as it is convenient to give in a single dose, and the resulting dilution when this enters the blood being necessarily extreme, very little effect can be anticipated. Still, successful and sometimes brilliant results are occasionally obtained after inoculations with bactericidal sera, and these results are difficult to understand if the accepted views on immunity are correct; it has, however, been suggested with much probability that such sera may have been produced by injecting animals with progressive doses of bacteria, following the method of injecting toxins in antitoxin formation. If this were done the animals so treated might well be in a state of "bacteræmia" and have organisms present in their blood, their serum would act as a vaccine, and any benefit

accruing from its use would be the result of active rather than passive immunisation.

These are the essential reasons for believing that for bactericidal purposes the attractive method of vicarious immunisation is impracticable, and that recourse must be had to vaccine therapy.

The contrast between the use of a chemical antidote, such as a serum, which cannot escape manifold dilution, and the use of a stimulus which will be followed by the production of antibodies in excess of the amount required to neutralise itself, is entirely in favour of the latter, when bactericidal action is required.

It is believed that in vaccine therapy we have a means of definitely attacking bacterial infection, which is both scientific in theory and satisfactory in practice.

Note.—For a full discussion of endotoxins and sera, see Emery, "Immunity and Specific Therapy"; and for the therapeutic value of sera, see Wright, "Studies in Immunisation."

CHAPTER III

VACCINE THERAPY IN PRACTICE. THE PROBLEM OF BACTERIOLOGICAL DIAGNOSIS

In the preceding chapters the theoretical basis of therapeutic inoculation has been briefly described, and an attempt has been made to give a simple account of the common sense of the method. As has been seen, it is based on two assumptions: first that recovery from bacterial disease is due to the formation of antibacterial substances by the tissues, and secondly that the tissues may be stimulated to a similar formation of antibodies for either prophylactic or curative purposes by the injection of dead bacteria in the form of vaccines, and that vaccine therapy is a rational method of treating disease due to bacteria which produce endotoxins.

The problem involved in the correct treatment of any given case by means of therapeutic inoculation remains to be considered. That problem may be resolved into the following parts:—the diagnosis of the infecting organism; the preparation of a vaccine; and the method of its administration, including dosage.

In the present chapter the bacterial infections are enumerated which have been most commonly treated by means of vaccine therapy. Stress is then laid on the necessity of accurately determining what organism is the

cause of any infection before treatment can be undertaken. Finally, the scope and limitations of clinical methods for such "bacteriological diagnosis" are discussed.

There is no theoretical reason why vaccines should not be successfully used against any disease due to an organism which produces endotoxins and is capable of artificial culture. It has been explained that localised disease offers better prospects of success than septicæmia. Even with that restriction it is clear that a very wide field of operation is open to this method, and much of this field still remains unexplored.

The curative treatment of bacterial disease by means of vaccines was first introduced in 1902, and for a long time progress was slow. Many difficulties have had to be overcome in the eight years which have elapsed, and the total number of organisms which have as yet been submitted to exhaustive experiment is not large.

The present volume is concerned only with well-established work, so that a brief survey is required of the pathogenic bacteria whose infections have been extensively treated by means of vaccines up to the present time.

A broad distinction is commonly drawn between pathogenic and non-pathogenic bacteria, a distinction which is not wholly justified. There are some bacteria which to the best of our present knowledge are always pathogenic, such for instance as the *bacillus tuberculosis* and the *bacillus typhosus*, but it is becoming increasingly difficult to say that any given organism is never pathogenic.

The essentially pathogenic organisms now recognised are far too many to enumerate, but those most commonly met with in practice are the following: *Cocci:* pneumococcus, meningococcus, gonococcus, and for practical purposes the various staphylococci and streptococci: *Bacilli:* B. anthracis, B. tuberculosis, B. diphtheriæ, B. lepræ,

B. mallei, B. typhosus, B. choleræ, B. pestis, Friedländer's bacillus, B. influenzæ, B. tetani.

When the specific treatment of infections with these organisms is considered, it is found that some have not hitherto been treated at all, some are commonly treated by means of immune sera, against others vaccines are employed for prophylactic purposes only, and a large number are left which have been extensively treated by vaccines for curative purposes.

Anthrax, glanders and leprosy, are all rare diseases and have only been treated in isolated instances if at all.

Diphtheria, tetanus, and meningococcus-meningitis are best treated by immune sera. The vaccine treatment of the last named is still experimental.

Typhoid, cholera, and plague are diseases against which vaccines are used for prophylaxis, chiefly in hot climates; the two latter are practically unknown in this country. The curative treatment of typhoid fever by vaccines is being experimentally carried out by officers of the Military Medical Services.

Infections with the remaining organisms, staphylococcus, streptococcus, gonococcus, pneumococcus, Friedländer's bacillus, influenza and tubercle have all been treated by means of vaccines, some of them to a very large extent. These are the purely pathogenic bacteria with which vaccine therapists are chiefly concerned and references will be found to most of these in the sections devoted to treatment.

The pathogenic nature of these bacteria is well known: disease resulting from infection with one or other of them is of common occurrence, and the attention of vaccine-therapists could not have failed to be directed to them. But there are a large number of other diseases which are bacterial in origin whose infective nature is commonly neglected; they are generally diseases of no great

severity, but chronic in course and highly resistant to treatment. These are perhaps most frequently catarrhal conditions of mucous surfaces, and a typical instance of this kind of infection is chronic bronchitis.

These infections are due in many instances to bacteria whose pathogenic nature is either not recognised or else neglected. For instance, in chronic bronchitis, a large variety of organisms may be found in the sputum of any patient which could also be found in the throats of healthy persons. These organisms are at first, and perhaps in most individuals, harmless parasites, which are liable to become pathogenic under some circumstances which are not understood.

Such bacteria are of very wide distribution under the conditions of ordinary life, and any surface to which either air or food have access is liable to become contaminated by them. That is to say, the whole of the skin, and the alimentary and respiratory tracts are exposed to such contamination. Certain kinds of bacteria show a preference for certain anatomical tracts, thus staphylococcus can always be cultivated from the skin, streptococcus from the mouth, and, best known of all, the bacillus coli from the large intestine.

The staphylococci and the streptococci are both instances of organisms which may at one time be harmless parasites and at another become pathogenic, though their pathogenic nature is so well known that they are commonly included in the essentially pathogenic group. All infections due to organisms of these types are amenable to treatment by means of vaccines.

Many of these organisms are as yet undifferentiated, and in the investigation of any case of the kind mentioned bacteria may be isolated which cannot be identified with any of those described in the text-books. These may be grouped in a rough and ready fashion, which, if not

scientific, is convenient. The organisms are stained by Gram's method. By this method the specimens are first stained with aniline gentian violet, and afterwards treated first with a mordant, and then with a decolorising agent, and finally counter-stained with a contrast colour. Certain organisms retain the violet stain and are called "Gram-positive," others lose it and take up the counter-stain and are called "Gram-negative."

Gram's method of staining separates bacteria into two large groups, much as red and black playing-cards may be divided. Just as in a search for the ace of clubs all red cards may be rejected, so in a search for pneumococci in a specimen stained by Gram's method all Gram-negative organisms may be neglected, since the pneumococcus is not decolorised.

The presence or absence of the power of retaining the stain when treated by Gram's method has nothing to do with the pathogenicity of any organism; the diphtheria bacillus and the pneumococcus are Gram-positive, the typhoid bacillus and the gonococcus are Gram-negative.

The bacteria likely to be found in these cases are chiefly cocci and bacilli, the more complex bacterial forms are comparatively rare. With regard to cocci, Gram-positive cocci are described, according to their grouping as staphylococci, streptococci, diplococci or tetrads. Cocci which are negative to Gram are generally arranged in pairs (diplococci), and when the gonococcus and meningococcus are excluded such diplococci are grouped under the title of *micrococcus catarrhalis*.

As to bacilli, Gram-positive bacilli of approximately the length of a tubercle bacillus, which are arranged irregularly, much as a handful of firewood might fall, are known as "diphtheroid" bacilli; while Gram-negative bacilli, which have rounded extremities, and stain more deeply at the poles leaving their middles clear, and which lie isolated a little

from their fellows, are included in the great group of "coliform" bacilli, of which the *Bacillus coli communis* is the type. Organisms of all these types may be found in health, and appear sometimes to become pathogenic.

If the alimentary and respiratory tracts are normally inhabited by bacteria which are potentially pathogenic, it is easy to understand how infective disease may arise in deeply seated organs. When there is infected mucus in the nose, organisms may pass into the nasal sinuses, through the Eustachian tube to the middle-ear, or through the cribriform plate to the meninges of the brain. From the small intestine bacteria can pass up the common bile-duct to cause cholangitis, cholecystitis and gall-stones, and the question of the infective nature of diabetes, intestinal organisms gaining access to the pancreas by way of Wirsung's duct, is not one to be lightly dismissed.

Clearly there is no theoretical reason why any one of these diseases should not be treated by means of vaccine therapy with a reasonable prospect of success. No over-statement is intended here. It is not meant that we are at present in a position to cure diabetes by a vaccine, but merely to suggest that from its position the pancreas is exposed to invasion by bacteria which may become pathogenic, and that if the disease can be found to be associated with infection by any particular organism its treatment can be attempted on more rational lines than has hitherto been possible.

Consideration of these points will serve to show the wide range of usefulness of vaccine therapy and the enormous field which has to be covered before its resources are exhausted.

The vaccine therapist is called upon to treat infections due to a large number of pathogenic organisms, which he must be prepared to recognise, and also many other infections due to bacteria which he can only differentiate into

large groups, and each of the latter cases must be treated on its merits.

For all who practise vaccine therapy the problem of diagnosis presents a new and formidable significance, and in emphasising this, it is hoped that a few platitudes will be pardoned. In the ordinary course of practice a patient presents himself for treatment on account of certain symptoms, and examination is made of the patient's cardiac, respiratory, and other systems by clinical methods, with a view to finding physical signs in one or more of them of conditions which will account for those symptoms. Thus, when a patient complains of shortness of breath, high temperature, and pain in the side, and presents the physical signs of consolidation of a lobe of lung, the diagnosis of "lobar pneumonia" covers all the essential facts of the case. When specific therapy by means of vaccines is not contemplated the question of whether the infection is due to the pneumococcus of Fränkel or the influenza bacillus of Pfeiffer is one of hardly more than academic interest, the prognosis may be better or worse in one case or the other, but no fundamental variations in treatment are adopted on that account. After arranging for the general management of the case, suitable drugs will be given for the relief of the respiratory distress, with proper regard for heart failure and other accidents. If, on the other hand, the patient is suffering from arthritis, whatever its nature, attention will be directed to the local condition, and rests, splints, massage, appropriate drugs, or similar means will be prescribed for its relief.

The exponent of vaccine therapy, pure and simple, finds himself in quite other circumstances. For him the diagnosis of "pneumonia," meaning an inflammation of the lung, is no diagnosis at all, his chief concern is with the infecting organism, for him "pneumonia" is an infection

with the pneumococcus, or the pneumobacillus or the influenza bacillus, and its seat in the lung is of quite secondary importance. Given such an infection he will employ the same means to combat it whether it is situated in the lung, the nasal sinuses, the meninges, the peritoneum or the knee-joint. In short, it is essential for him to make a *bacteriological* diagnosis, for vaccine therapy is applied bacteriology.

It will be seen that the practitioners of clinical medicine and specific therapy may easily become widely divergent in their methods, and how essential it is to bring these into accord for the benefit of the patient. The object of the clinical treatment of infective disease, whether avowed or unconscious, is to support the patient's strength and relieve his symptoms until such time as he shall have made his own antibodies and established his own immunity. It is to this end that the patient is placed in the most favourable circumstances possible, and the various methods are employed to relieve pain, induce sleep, promote expectoration, stimulate the heart and so on, as may be required. It behoves the vaccine therapist to remember that all these things are as essential when his methods of treatment are adopted as in any other case, a matter which is not infrequently forgotten.

But these methods produce no direct effect on the infecting organisms. We have no clinical means of attacking bacteria in the tissues. Antiseptics are available only against such organisms as they can be brought into contact with, all attempts to introduce them into the circulation have failed, no drugs given by the mouth have any influence upon bacteria at all. Without bacteriotherapeutic methods we are quite powerless to influence in a direct manner the pathogenic bacteria causing disease. But it is very clear that vaccine therapy should be used in conjunction with and not to replace the clinical methods of treatment.

The work of the vaccine therapist begins when clinical methods have revealed in some organ a lesion which is suspected of being bacterial in origin. The identification of the infecting organism is an essential without which he cannot undertake any treatment by specific methods. If, for instance, he attempts to treat a gonococcal arthritis on the assumption that it is tubercular, and so proceeds to immunise his patient against the tubercle bacillus, he will do no more good to the arthritis than if he were to immunise the patient against sheep's corpuscles.

The diagnosis of bacterial infections may be made either by the clinical method, that is, by observation of symptoms and physical signs, or by strictly bacteriological methods; the latter will be discussed in the succeeding chapter.

It is first necessary to consider how far any combination of symptoms and physical signs justifies the opinion that a given lesion is the result of infection with one specific micro-organism and no other. Such opinions are frequently formed, and doubtless are frequently correct. For instance a cutaneous furuncle is considered by clinicians and vaccine therapists alike to be a staphylococcal infection. A lesion at the apex of one lung, associated with local wasting, crepitations and signs of consolidation, with night sweats and a remittent temperature would be diagnosed without hesitation by all clinicians as tubercular. Tubercular lymphatic glands, and other tubercular manifestations are diagnosed with confidence every day. The "steppage" temperature chart, the rash, the abdominal symptoms and the stools of typhoid fever are characteristic. It may be stated that a clinical diagnosis of the bacteria present in any lesion can only be made on the strength of numerous past examinations of similar cases, in which the symptoms or physical signs have been so regularly associated with certain bacteria that the exceptions may be neglected. The advantages of such a method, when applicable, are its

speed and its freedom from trouble, its disadvantage is that it rests on opinion and not on fact. The saving of time is a very great consideration to all persons engaged in bacteriological work, and if accurate diagnosis can be made by clinical means, it is of great advantage to all concerned.

But though the symptoms of typical typhoid fever are nearly always associated with the presence of the *bacillus typhosus* in the stools, it is surely a very large statement that such symptoms *cannot* be produced by any other organism. That indeed is not the case, for the paratyphoid bacillus may produce symptoms which are clinically indistinguishable from those of typhoid fever, and bacteriological workers will feel similar doubts with regard to the infallible connection between any group of symptoms and a specific organism. The attempt at bacteriological diagnosis by clinical means is a question which requires very careful consideration. For purposes of inoculation, bacteriological diagnosis is not a mere matter of opinion, but one which profoundly influences action, and an error means a period of incorrect treatment, which can by no possibility result in the slightest benefit to the patient. This risk ought to oblige vaccine therapists to exhaust all available means of diagnosis, for even with that precaution error will occur with quite sufficient frequency.

Can any practical use, then, be made of clinical diagnosis? It depends on the nature of the organism and of the lesion. Inoculation with some vaccines produces clinical results very quickly, in others changes are produced only very slowly. In superficial infections, such as boils, clinical results are quickly manifest, and the results of incorrect dosage are less serious than in deep seated lesions. For instance, in the treatment of a boil, after an inoculation with staphylococcus vaccine there is frequently a marked change in the condition within a few hours. If the boil gets well the dose was correct, if the boil gets rapidly worse the dose

was too large. No bacteriological investigation will give clearer information than this, and the consequences of incorrect dosage are so transient that the physician may fairly accept the risk. But in such a condition as chronic arthritis in which no clinical change may be manifest for a long period, the circumstances are quite different, and the physician is hardly justified in proceeding without some guide as to whether he is doing right or wrong.

It has been found by practical experience up to the present time that certain infections of the skin such as furunculosis, erysipelas and acne, may be treated with fair confidence under purely clinical control. This applies equally to other infections of which individuals happen to have had special experience. Many tubercular lesions may be diagnosed clinically, with sufficient certainty to justify the initiation of treatment, and many cases may be brought to a successful termination by treatment guided only by clinical indications, but there are numerous instances in which serum reactions are required both for diagnosis and for control of treatment, and they certainly cannot be abandoned. In nearly all other cases definite bacteriological investigation is required.

The bacteriological infections therefore which at present come within the scope of vaccine therapy are very numerous, and the infecting organisms include some of well-known pathogenic virulence and others which are sometimes pathogenic and sometimes harmless parasites. It is absolutely essential for correct bacteriological diagnosis to be made before treatment is initiated, and for this purpose clinical methods are of very limited application.

CHAPTER IV

THE ACCURATE METHODS OF BACTERIOLOGICAL DIAGNOSIS

CORRECT bacteriological diagnosis is the first essential in the practice of vaccine therapy, and since clinical examination is of very limited value for this purpose, the more exact methods of identifying pathogenic organisms must be considered.

When a lesion is discovered by clinical means which is suspected of being bacterial in origin, there are two distinct methods by which the question may be investigated. The first method is to obtain the bacteria themselves from the lesion, and the second is to test the patient's blood for antibodies. These methods may be used separately or in combination according to circumstances. The two methods may be briefly styled "diagnosis by demonstration" and "diagnosis by serum reaction."

The method of direct demonstration will suffice for diagnosis in two cases: first, when a definitely pathogenic organism is obtained; for instance, when tubercle bacilli are found in sputum, tubercular disease of lung or larynx is conclusively proved: secondly, when some organism is present in large numbers; for instance, in the examination of pus, the presence of a few staphylococci together with other organisms is not diagnostic of a staphylococcal infection, since these bacteria are always present on the skin, but

when they are prevalent throughout the specimen and other organisms are absent, that is regarded as sufficient proof that they are the cause of the lesion. Caution is required in examining specimens taken from sites where a particular organism is normally present in very great numbers, for instance, little information is received when a specimen from the large intestine is found to be swarming with *B. coli*.

This method of direct search is limited to "open" lesions, or lesions in sites accessible to direct examination.

Diagnosis by unaided serum reactions may be practised when a lesion is suspected on clinical grounds of being due to any organism of which a laboratory culture can be obtained. Diagnosis is by this means a process of exclusion and is limited to the common pathogenic bacteria. It is most commonly used to check clinical diagnosis in a limited number of infections, of which tuberculosis is the chief.

The methods of direct demonstration and serum diagnosis may be combined in cases in which more than one organism is present in any lesion. Serum tests are applied to all the organisms obtained in order to pick out the cause of infection, often a most laborious process in practice.

In selecting the method of diagnosis the following points must be borne in mind. Diagnosis by direct demonstration is limited to cases from which pathological material is obtainable. Diagnosis by serum reaction is limited to organisms of which cultures are obtainable.

In any given case it may be convenient to select serum reactions in preference to direct demonstration, when the latter would be possible. For instance, in a suspected case of typhoid it is preferable to perform an agglutination test than to isolate typhoid bacilli from stools and identify them by fermentation reactions.

The choice may also be influenced by other considerations.

Some cases may be treated by ready-made "stock" vaccines, in others special vaccines made from organisms isolated from the patient's own lesion are required. Stock vaccines are as a rule only obtainable for infections by the commoner pathogenic bacteria. Diagnosis by serum reactions may be used in such cases, but when special vaccines are required, diagnosis by direct demonstration is necessary, since the bacteria themselves have to be obtained for making the vaccine.

On the other hand, with organisms of doubtful nature identification is merely a matter of nomenclature, and it is preferable to proceed to make vaccines direct from the organisms obtained than to make any attempt to identify them.

Diagnosis by Demonstration.—This method, as was stated above, is limited to lesions from which bacteria can be obtained. Lesions are described as either "open" or "closed," and with rare exceptions it is only from "open" infections that bacteria can be isolated.

"Open" infections are those from which pathological material can be directly obtained. They include lesions of the skin and certain mucous membranes; lesions of deep organs accessible through the skin by means of operation wounds or sinuses; lesions of mucous surfaces which, when infected, produce secretions which escape by patent passages or are periodically expelled, such as the nose, lungs, or vagina; lesions of excretory organs discharging externally, such as the colon and the urinary system.

"Closed" infections are those situated in deep organs, such as the heart or the central nervous system, which do not communicate in any manner with the exterior. In some cases of closed infection direct search may be made for bacteria, namely those in which pathological material can be withdrawn by aspiration. This is really rendering the "closed" lesion "open" by operation. Thus

septicæmia may be investigated by withdrawing blood from a vein and making cultures therefrom, and effusions into serous or synovial cavities, such as the pleura, peritoneum, knee-joint or spinal theca may be drawn off and examined in the same way.

Closed infections, however, are not always wholly out of the reach of diagnosis by demonstration. In any infective lesion of a deep-seated organ the bacteria have entered through some breach in the skin or mucous membrane and search should be made for any accessible septic focus from which bacteria may be obtained. Such foci are frequently found in the mouth, and of late years the importance of oral sepsis as a cause of disease has received a good deal of recognition.

Moreover, infecting bacteria often escape from a lesion and leave the body by way of the excretory organs, and may sometimes be recovered from the urine or fæces. Organisms so obtained may be subjected to the serum tests, or vaccine treatment may be attempted on empirical lines.

Some practical points in the treatment or examination of specimens for bacteriological diagnosis require emphasis.

This is not the place for detailed description of the methods to be employed in the examination of pathological material, it is to be found in all text-books on bacteriology, and only enough will be given here to render the steps intelligible to those who do not practise this branch of medicine.

When a specimen is obtained for bacteriological examination part of it is examined under the microscope, and part is planted out on to suitable media for cultivation of its bacteria.

Films are made from the material for microscopic examination and stained by Gram's method, and if it is necessary to exclude tuberculosis, further films are stained by the Ziehl-Neelssen method. In examination of such

films two objects must be kept in view. The first is the identification of any pathogenic organism which is recognisable at sight, the second is the observation of any organism which is predominant in the specimen.

In many pathological specimens a large number of different organisms may be seen, but on culture very few varieties will be obtained. Attempts at artificial immunisation can, however, only be made against such organisms as are capable of cultivation, so that in such cases it is generally necessary to be guided by the organisms obtained from cultures.

The first object of search is recognition of any purely pathogenic organisms in the specimen.

The difficulty of this investigation varies very much. Sometimes pathogenic bacteria are easily recognised, this is the case with the tubercle-bacillus from its shape and peculiar staining reactions. Others, such as the diphtheria bacillus, the influenza bacillus, and the pneumococcus may often be identified with confidence when present in specimens of certain kinds, for instance, the presence of bacilli with the morphological and staining properties of the diphtheria bacillus would be conclusive of diphtheritic infection if present in a piece of false membrane, but such infection would be quite open to doubt if such bacilli were found in the pus from a case of pyorrhœa alveolaris, when they would probably be "pseudo-diphtheria" or "diphtheroid" bacilli.

When the most prevalent organism in any film is sought, the difficulties will vary considerably with the nature of the specimen. In the case of pus from an abscess, the presence of abundant staphylococci will be sufficient for diagnosis, and frequently no contaminating organisms will be found; but if a specimen be taken from one of the mucous tracts to which air or food have access, more especially if it be from the mouth, into which many other things are intro-

duced, bacteria of all sorts and kinds will be present, and it will be very hard to decide which is the predominant organism. These are the cases in which attention has to be directed rather to the cultures than the films. Pus from pyorrhœa alveolaris is one of the extreme cases of difficulty in this matter.

In such pus Goadby has described twenty organisms as commonly present, and a glance at a film with its nearly infinite variety of bacillus, coccus, streptothrix, comma and what not, may well compel the opinion that it is a matter of great good fortune if the infecting organism can be isolated and cultivated. A less extreme case, and a more profitable investigation is afforded by sputum from the lungs. Herein may be found pneumococci, streptococci, staphylococci, diplococci, diphtheroid, coliform and cocco-bacilli, and it may be impossible to decide upon the prevalence of any one of them, and previous experience is of considerable help in selecting that most likely to be infective. In such a group, for instance, the pneumococcus, the cocco-bacillus of influenza, and Friedländer's coliform-bacillus would come under suspicion.

The importance of examining films is for the detection of pathogenic or prevalent organisms. The culture of the organisms now requires consideration.

Having examined the film and come to some conclusion as to the organism most likely to be the cause of infection, cultures are made upon the media most suitable for its isolation. Agar is the medium in common use; but if the influenza-bacillus is suspected blood-agar is used, or if the pneumococcus, serum-agar, and so on. If coliform organisms of more than one variety are present, it may be necessary to isolate and identify them; this is done by obtaining sub-cultures from single colonies and planting them into a series of broth tubes containing litmus and various sugars in solution, and provided with fermentation

tubes. The different members of the *B. coli* group may be identified by their power of fermenting some sugars and not others, each member having affinity for certain sugars only: for instance Friedländer's bacillus ferments dulcite, glucose, lactose, mannite, and saccharose, *B. coli* ferments all the above, except saccharose. No attempt is made to obtain cultures of the tubercle bacillus.

Pathological fluids obtained from closed lesions by aspiration are treated in the same manner.

Blood cultures are obtained from cases of septicæmia by puncture of a vein with a hypodermic needle attached to a sterile syringe and aspiration of ten cubic centimetres of blood. This is divided among half a dozen broth tubes, which are incubated and tested for organisms. Films are generally omitted in blood culture cases and reliance is placed only on the growth obtained.

Blood cultures are rarely successful if the patient's temperature is less than 101° F. The large number of tubes used and consequent dilution of the blood are necessary in case of the presence of antibodies in the blood which may inhibit growth unless much diluted.

If the evidence for the infective nature of any organism is sufficiently strong, it is obtained in pure culture, and a vaccine made forthwith, but if there are more than one present, and decision between them is impossible, diagnosis must depend upon investigation of the patient's serum reactions.

Diagnosis by Serum Reactions.—The serum of healthy persons normally contains antibodies to each of the pathogenic bacteria, and the amount of antibodies to any organism in a patient's serum is modified in the course of an infection due to that organism. The diagnosis of the organism causing any given disease may be made by means of serum reactions. For this purpose an estimation is made of the antibodies in the patient's serum to any organism suspected of being the cause of infection.

Several different antibacterial reactions can be demonstrated with serum, some of which are qualitative, and others quantitative. In the case of some organisms, the amount of antibody which can be demonstrated in healthy serum is small. In these cases a test which clearly reveals the presence of antibody in a suspected serum, when it reveals none in a healthy serum, indicates that a large amount of antibody is present, and this is sufficient for diagnosis. But in cases where the demonstrable differences in antibody between normal and infected serum are small, quantitative tests are required.

The best known antibacterial reactions demonstrable with serum are the bactericidal reaction, bacteriolysis, agglutination and opsonization, the latter two of which are most widely employed in practice.

For the performance of all these reactions a culture of the suspected organism is required.

The bactericidal properties of serum on the typhoid bacillus have been previously referred to. Bacteriolysis is demonstrable against cholera vibrios. Both of these are long and difficult experiments, and of limited practical application, they need not be described in this book.

Agglutination is a reaction in frequent use, chiefly against the *bacillus typhosus* and the *micrococcus Melitensis*. When employed as a test for typhoid fever it is known as the "Widal reaction." It is commonly employed as a qualitative reaction only. It depends on the fact that the serum of a person who has responded to infection with typhoid acquires the property of "clumping" a suspension of typhoid bacilli, that is of collecting the bacilli together in masses large enough to be visible to the naked eye. It is performed by mixing equal volumes of a suspension of the bacilli and the suspected serum, incubating the mixture at 37°C. and observing the agglutination either with the naked eye or under the microscope. As a rule the experiment is repeated

with equal volumes of progressive dilutions of the serum with salt solution. The reaction should be complete within half an hour.

Agglutination is a satisfactory diagnostic test in the infections mentioned. There are two fallacies which require caution. These will be evident when it is remembered that agglutinins are present only in small amounts in normal blood and are evidence of resistance to the infection. The consequence is that in grave and fatal cases where there is little effort at recovery the reaction may be absent, and also that in cases of recovery the reaction persists throughout the succeeding period of immunity so that such a reaction may be due to a past and not a present infection.

The opsonic reaction however is one of far wider application than that of agglutination. It is concerned with the influence of serum on the phagocytosis of bacteria by leucocytes. It is well known that leucocytes, the white corpuscles of the blood, have the power of ingesting foreign substances in general and bacteria in particular, a power which will be made evident by the examination of gonorrhœal pus, when many gonococci will be seen ingested by the leucocytes. This property of ingesting foreign bodies is known as "phagocytosis." The opsonic reaction concerns the influence of serum on the phagocytosis of bacteria. For its investigation leucocytes are required free of all serum, the method of their preparation is described in the appendix.

A pure culture of the suspected organism is obtained, and a thin emulsion is made with salt solution. Equal volumes of bacterial emulsion and blood corpuscles are now mixed and aspirated into a capillary pipette, and the mixture is incubated for fifteen minutes. After this a film is made of the mixture, which is fixed, stained and examined. It will be found that no phagocytosis has occurred, or very little.

The experiment is now repeated, adding to the equal

volumes of bacteria and corpuscles a third equal volume of healthy serum. After incubation it will be found that phagocytosis has occurred to an extent which varies with the strength of the emulsion, and an average per cell can be worked out.

To reconsider the concrete instance suggested in Chapter II, suppose an emulsion of tubercle bacilli is employed, and that it is of such strength that if a volume of it is incubated with equal volumes of a healthy person's serum and of washed corpuscles, the leucocytes ingest in fifteen minutes an average of five bacilli per leucocyte. If the experiment is repeated, using the serum of other healthy persons, there will be phagocytosis of not less than four or more than six cocci per leucocyte. That is to say, approximately the same degree of phagocytosis will result whatever serum is used, provided it is that of a healthy person. The substances in the serum which act upon the bacteria in such a way as to prepare them for ingestion have been called "opsonins" (*ὀψωνῶ*, I prepare for the table). The blood of healthy persons contains anti-tubercular opsonins in approximately equal amounts.

If the experiment is repeated, using the serum of a person who is suffering from tubercular arthritis who has been kept strictly at rest for forty-eight hours, his serum will be found to contain less opsonin than healthy serum, so that the phagocytosis resulting will average perhaps three cocci per leucocyte (*see* Chart 8).

In recording such an event, it is usual to express the average phagocytosis obtained with normal serum as unity, and to express that obtained with the patient's serum as some fraction or multiple of one. The antibacterial power of the patient's serum is therefore expressed in terms of the normal. Thus if normal serum yields an average of 5 bacilli per leucocyte, and the patient's serum an average of 3 bacilli, the normal result is regarded as unity

and the patient's as $\frac{3}{5}$ or 0·6. This figure is called the patient's *opsonic index* to tubercle. It has been explained in Chapter II that if, in such a case, the infected joint is subjected to hyperæmia auto-inoculation may be set up, bacilli may be washed into the circulation and may lodge among tissues capable of forming antibodies, and that the antibodies may be exalted or depressed according as the dose thus given is large or small. It will then be found that the opsonic index has risen or fallen in response.

In Chapter I it was assumed for the moment that all healthy sera contain antibacterial substances in approximately equal amounts. That statement now requires qualification. It appears to be strictly true of tubercle and gonococcus, two of the purely pathogenic bacteria, but sufficient work has not yet been done on the others to allow of positive statements. With regard to the potentially infective bacteria, it is probably not true, as indeed is unlikely from the widely varying susceptibility of individuals to infections with such organisms.

It is commonly agreed that there is considerable disparity in the opsonic content of the serum of healthy persons to these organisms, and when dealing with them it is usual to employ as a control the pooled serum of about six normal individuals. The limits of health are much wider with these organisms than with tubercle, but any grave deviation from the average, as indicated by the pool, is considered to indicate infection.

It does, however, appear to be true that the opsonic index of an uninfected person to all bacteria is *constant*, whether it is above or below the average, and it is not influenced as the result of rest or exercise. Thus in the case of gonococcal arthritis, no amount of hyperæmia of the site of infection will wash tubercle bacilli into circulation, so that while the opsonic index to gonococcus will vary, that to tubercle will remain unchanged.

This method of induction of auto-inoculation is the most valuable serum test which is available for bacteriological diagnosis. Given an infection such as an arthritis which may be tubercular or gonococcal in origin, and which cannot be decided upon on clinical grounds, the evidence of auto-inoculation with one or other as a result of local hyperæmia is sufficient indication of the vaccine which it is necessary to employ.

The estimation of the opsonic index is the most generally applicable of the serum reactions, but only wide deviation from the normal or evidence of auto-inoculation is to be regarded as diagnostic. When any lesion is diagnosed as the result of a particular infection on clinical grounds it may be submitted to this test, and in the case of an infection from which more than one potentially infective organism has been isolated the opsonic index may be used to discriminate between them.

The opsonic index is really but a rough method of investigation. It is lengthy, laborious, and involves several processes each of which allow of errors in technique. It requires considerable skill, experience and conscientiousness. The information it gives is frequently inconclusive.

But in many instances it does give information of extreme accuracy and value, and even when it merely suggests it gives a certain amount of information, and imperfect though its guidance may be, it is by far the best method which is at present available for the serum diagnosis of the majority of bacterial diseases, and until some better method is evolved this will have to hold the field.

The problem of the bacteriological diagnosis of infective disease may therefore be solved by one of three methods, namely, by the clinical characteristics of the lesion, by the demonstration of bacteria in material collected from the lesion, or by some serum reaction, which is generally the presence

of an abnormal opsonic index or variation in the opsonic index induced by hyperæmia at the site of the lesion.

Clinical methods are applicable in superficial infections of common occurrence and familiar appearance. Bacteria may be isolated from open infections, and when many varieties are found choice must be guided by the recognition of well-known pathogenic bacteria, or by the presence of bacteria of potential infectivity likely to be infective in a given site, or by estimation of the opsonic index to all the bacteria isolated. Serum reactions are applicable in cases of closed infection where the clinical conditions are such as to suggest infection by one or more well-known pathogenic bacteria, but whose appearance is not sufficiently typical to justify an unaided diagnosis.

CHAPTER V

THE PREPARATION AND USE OF VACCINES

Vaccines may be successfully used in the treatment of any disease which is due to an organism which produces endotoxins, and is capable of artificial cultivation. When an organism has been cultivated, and identified as the cause of an infection on direct bacteriological evidence or by means of serum reactions, a vaccine may be prepared from it.

In the following pages the principles involved in the preparation of a vaccine are stated, with some generalisations on the method of administration, and a note on the clinical precautions which should accompany this method of treatment. The technical details are described in the appendix.

A vaccine, as has been said, is a sterilised suspension of bacteria of known strength. For this purpose a pure culture of the organism is required, preferably on a solid medium. The medium which is the most generally applicable and the easiest to work with is agar.

A few cubic centimetres of one per cent. sterile salt solution is poured on to the agar, and the colonies are rubbed off the surface of the medium, and so suspended in the salt solution. The suspension of bacteria thus made is poured

into a sterile test tube which is hermetically sealed. The tube is shaken, either by hand or some mechanical contrivance, till the bacteria are evenly distributed through the solution.

The tube is opened, a sample is withdrawn for standardisation, the tube is closed again, and sterilised by immersion in water at 60° C. for a suitable period.

After this it is again opened, a second sample withdrawn, and the tube again closed. The fresh sample is planted out on agar and incubated for twenty-four hours. If no growth appears, the sterilisation of the vaccine has been efficient, and it may be used for inoculation.

Standardisation.—The vaccine at this point is a suspension of organisms of unknown strength. It is required to estimate the number of organisms present in each unit of volume. The estimation is made by thoroughly mixing the bacterial suspension with a suspension of other bodies of known strength in certain proportions, spreading the mixture on a flat surface and comparing the numbers of bacteria, and of the elements of the other suspension which are present in a given area. From the figures obtained the comparative strength of the two suspensions is found out, and since one is known, the absolute strength of the unknown one can be calculated.

The standard suspension of other bodies used is normal blood, which is a suspension of corpuscles in serum. A volume of blood is mixed with one or more equal volumes of vaccine, and the mixture is spread on a slide, which is stained and examined microscopically. The number of red blood corpuscles and bacteria in a series of fields are counted, until a total of 500 red corpuscles with the corresponding organisms has been reached.

The calculation is made as follows:

500 r.b.c. (red blood corpuscles) = x bacteria.

$\therefore$ 5 million r.b.c. = x × 10,000 bacteria.

But 1 c.mm. blood contains 5 million r.b.c.

∴ 1 c.mm. of vaccine contains x × 10,000 bacteria.

∴ 1 c.c. vaccine contains x × 10,000 × 1,000 bacteria.

Vaccines so made are generally too strong for immediate use and require to be diluted. For this purpose sterile 1% salt solution, containing ½% phenol is employed. The suitable strength varies with the organism used, so that different degrees of dilution are required in different cases, and when diluted the vaccine is stored either in rubber-covered bottles, if large quantities are required, as for hospital use, or in blown-glass capsules or ampoules, containing about 1 c.c., for individual cases.

Such are the principles of the preparation, sterilisation, standardisation, dilution, and storage of vaccine.

A vaccine thus prepared may be used as a "stock' vaccine for the treatment of infections where a special or "autogenous" vaccine is not required. Many infections can be treated quite successfully in this way, but others always require special vaccines. As a rough guide it may be stated that infections due to essentially pathogenic bacteria, including staphylococci, may be treated with stock vaccines, but infections due to potentially infective organisms generally require special vaccines. Sometimes cases of the former type fail to respond to stock vaccines, but improve rapidly with autogenous vaccines, and doubtless many other exceptions would be found to any such rule, but as a rule of thumb it will be fairly dependable.

Another reason for making autogenous vaccines for potentially pathogenic organisms, is that it will often be found shorter to make a vaccine from a given organism than to establish its identity with one for which a stock vaccine is available, this is especially true of organisms of the bacillus "coli" group.

Vaccines thus prepared are intended to be used as a stimulus to the tissues to form antibodies in excess of the

amount required to neutralise them, so that the excess may pass into the blood and lymph, and be available against living bacteria which are causing infection in any part of the body.

For this purpose they must be introduced among the tissue whose function it is to make antibodies. These cells are doubtless of very wide distribution, but they are specially to be found in the subcutaneous tissues; it has been experimentally shown that an immunising response is far more readily called out by subcutaneous than by intraperitoneal or intravenous inoculation.[1] Certainly, the subcutaneous tissue is the site which is most accessible to therapeutic inoculation, and on reflection it will be seen that there may be a definite physiological reason for this. The skin is always liable to abrasions and other breaches of surface through which bacteria may enter, and it is of the first importance that the organisms shall immediately come into contact with cells capable of providing antibodies to them. If it is a physiological function of the tissues to form antibodies to bacteria, it is but natural for the cells which subserve that function to be placed where they will be of the most use, that is in the subcutaneous tissue.

Some exponents of vaccine therapy commonly give vaccines by the mouth, and it is claimed that equally good results are obtained thereby. The alimentary tract is another channel of infection, and doubtless this is also well supplied with tissues having the function of forming antibacterial substances. It is difficult, however, to see that the oral method of administration has any advantage over the subcutaneous. The great advantage of the latter method is that a precise dose is introduced directly into the tissues which form antibodies, while by the former a dose is

[1] Noon, "Evolution of Immunity . . . in Disease." Journal of Hygiene. 1909.

received into the stomach, and whether any of it is absorbed or not is entirely a matter of chance. It is argued that patients object to the use of the needle: this argument has not much to support it. If a fine needle be used, and if this is clean and really sharp, the operation is acknowledged by patients to be the merest trifle; children are inoculated over and over again without demur.

The subcutaneous tissue, therefore, appears to be the best place for the introduction of vaccine. Any part well covered with fat may be selected. For vaccines which do not produce much local reaction, the back of the humerus is convenient, for others either the skin of the abdomen or that over the supraspinous fossa are good places, and when reaction may be considerable the loose tissues just below the clavicle are very suitable. The back should not be chosen for patients confined to bed.

The injection is given with a hypodermic syringe and the best for the purpose is one which has a glass barrel with metal plunger and fittings. Any syringe used must be carefully sterilised, and the most efficient method of so doing is to fill the syringe with any hot vegetable oil, olive oil is the most convenient. The oil should be heated in a metal vessel to about 140° C., a temperature very much above that of boiling water. If no high temperature thermometer is available, a bread crumb may be put in the oil. When the bread turns brown the oil will be sufficiently hot. If the syringe is filled two or three times with oil at this temperature it may be used with perfect safety. If the oil is used again after inoculation, the needle will not rust, and no wire need be passed through it. It has been pointed out that the needle must be kept thoroughly well sharpened; a small oil-stone is required for this purpose.

The skin, if reasonably clean, need not be specially

prepared. It is usual to smear the site chosen for inoculation with lysol, which must be wiped off again immediately the needle is withdrawn, or the skin will be burnt.

It has been seen that only certain limited doses of vaccine call forth a satisfactory immunising response, and that excessive doses do not stimulate, but depress the formation of antibodies, hence the question of dose is one of extreme importance, yet it is one about which it is difficult to generalise. Doses vary from one million gonococci, for the treatment of an acute infection, to two thousand million typhoid bacilli for prophylactic purposes. Dose has therefore to be considered as a separate problem with each organism.

The most scientific method of investigating the subject is to observe the effects of various doses upon the opsonic index and to select one which produces a short and slight negative phase, followed by a well-marked rise. Much work has been done on these lines with regard to infections with tubercle, gonococcus and staphylococcus, and the outcome of it is that there are very great variations in susceptibility dependent on the site and character of the lesion, whether acute or chronic, extensive or small, and further variations depending on the individual.

The first and most important rule of dosage is that the larger and more acute the lesion, *the smaller must be the dose*, and *vice versâ*. Another general rule is that when a suitable initial dose is found it should be gradually increased, but no dose should be increased till it has ceased to produce an immunising response.

The following figures will give a rough idea of safe initial doses of the common vaccines: Staphylococcus, 100 million, streptococcus and gonococcus, $2\frac{1}{2}$ million; pneumococcus, Friedländer's bacillus, influenza, 10 million; tubercle 1/20,000 mg.

Doses are commonly given at intervals of from seven to ten days, the latter has become the established interval between tubercular inoculations. For more acute infections it may with advantage be reduced to intervals of three days, a small dose being given; this must only be done with doses which do not produce a negative phase. Further questions of dose must be deferred till the treatment of the various lesions is discussed.

The phenomenon of auto-inoculation, the fact that bacteria may be washed out of a focus, and lodging in other sites may there induce antibody-formation, has been described, and the necessity for controlling auto-inoculation has been pointed out. But there are some cases which, in spite of inoculation and increase in the opsonic index, do not improve clinically. In these cases local hyperæmia may be induced in order to bring the blood, enriched in antibodies by inoculation, to the site of the lesion. For this purpose the affected part is massaged, heated, exercised, or passively congested. Cases for such treatment must be selected on their individual merits.

It has also been insisted upon that vaccine therapy is to be used as an adjuvant to, and not in place of, clinical methods of treatment.

Any lesion undergoing treatment by vaccine therapy must be regarded in a perfectly surgical spirit, at any rate in a spirit of conservative surgery. If a surgeon has to treat a case of enlarged glands in the neck, he first searches for, and removes, any source of irritation, such as pediculi capitis, carious teeth or dead bone in the mastoid. The same must be done in treatment by vaccines.

The sequence of events which terminates in a tubercular cervical gland is often as follows :

There is a carious tooth from which some septic material is absorbed by the lymphatics and lodges in a cervical lymphatic gland. The gland becomes inflamed, the cells

are damaged, and if tubercle bacilli become lodged in it the damaged cells cannot elaborate antibacterial substances. The tubercle bacilli may be removed by inoculation treatment, but so long as the source of irritation is present in the jaw the gland is liable to be in a state of adenitis, and reinfection may easily occur. These sources of irritation are foreign substances, over which vaccines have no influence. They harbour bacteria, and since there is no circulation of body fluids through them these bacteria are not exposed to antibacterial substances, and are always able to reinfect the lesions.

It was pointed out in the second chapter that fluids drawn from the site of infection are poorer in antibodies than the circulating blood. Whenever stagnant fluids are detected in the neighbourhood of a bacterial lesion they should be removed. From the point of view of the vaccine therapist the best method of removal is by puncture and aspiration. By this means a negative pressure is produced at the site of infection, so that lymph is drawn from the peripheral tissues thereto, and this lymph, as has been shown, especially after inoculation, is richer in antibodies than that which has been withdrawn. The removal of stagnant fluids is therefore an essential part of rational treatment by vaccine therapy, because it induces a flow of antibacterial lymph to the site of infection. When the pus has been removed from an abscess by aspiration or incision, or when such conditions as chronic sinuses from which there is little serous discharge require treatment, a flow of lymph may be induced through the tissues by chemical means.

If a solution of 5 per cent. sodium chloride and 0·5 per cent. sodium citrate is applied to lesions of these kinds, in fomentations, the coagulation of the plasma is retarded by the presence of the citrate, which combines with the calcium, and the salt solution, which is stronger in

sodium chloride than the plasma, induces a flow of fluid to dilute the salt, thus maintaining an outward flow of lymph through the lesion.

Pus, then, must be removed from abscesses, stones from bladders, sequestra from ulcers. For the present it is enough to state that many of the failures attributed to vaccine therapy are due to neglect of this principle.

For the preparation of vaccines, then, bacteria are obtained in pure culture, emulsified in 1 per cent. salt solution, sterilised by heat at 60° C. for an hour, standardised by numerical comparison with human blood, and diluted to suitable strength with $\frac{1}{2}$ per cent. phenol in 1 per cent. salt solution. When used they are injected into the subcutaneous tissues with a hypodermic syringe in doses too variable for generalisation, but which should err rather on the side of being too small than too large when there is any doubt as to the correct dose. They are generally given at intervals of about a week. Consideration is required in each case as to whether hyperæmia of the affected part should be avoided or induced, and finally all source of irritation must be removed from the neighbourhood of the lesion.

CHAPTER VI

RESULTS OF THE TREATMENT OF BACTERIAL DISEASE BY VACCINES

On theoretical grounds the case for vaccine therapy is fairly well established, but between theory and practice there is often a great gulf fixed, and the question which concerns practitioners of medicine is how far its promise is borne out in performance. An attempt will therefore be made to estimate the value of the method by consideration of the actual results obtained.

To form such an estimate is by no means an easy matter.

The proper method is to consider two large series of cases, treated under similar conditions, one of them by means of vaccines, and the other on an alternative plan. Such series are not available. Another method is to consider a series of selected severe cases which have done well. Description of such cases shows what results may be obtained by this treatment, and is an implied challenge to the rest of the world to produce like results. This, on the whole, is not a satisfactory method. Every treatment which has anything to recommend it at all produces some brilliant results, and that every case succeeds under any form of treatment is a claim made only by charlatans. In the absence of any proper standard of comparison it appears best to give a bare summary of the results of treatment of the infections

of which there is a large experience and sufficient records.

Before beginning the consideration of such results it will be well to define clearly what measure of success is within the compass of vaccine therapy, and what pathological matters are altogether outside its scope. The result of successful inoculation with a vaccine is the production of specific antibodies to the corresponding bacteria. This will help to destroy those bacteria when they are present in the tissues, and so situated that the tissue fluids can get access to them, and that is all. When a given infection simply produces an inflammatory condition, as in subcutaneous staphylococcal infection, before the formation of pus, nothing but an increase in antibodies in the blood is required. But all cases of infection are by no means so simple as this. When the staphylococcal infection has continued for some time, an abscess forms, containing pus, which consists of dead cells in a fluid which is poorer in antibacterial substances than the circulating blood. Is the development of antibodies likely to get rid of this pus? If the focus is a small one the bacteria may all be killed and the pus may be afterwards absorbed, but if absorption occurs it is due to other properties than those with which vaccine therapy is concerned. If pus is present it must be let out; it is asking altogether too much of a method which is concerned solely with the cause of disease, to call upon it to remove the effects. But, the pus once removed, healing will be hastened by inoculation, which will assist in the removal of bacteria from the living tissues.

In tubercular infections also, highly poisonous toxins are formed and necrosis frequently occurs in the neighbourhood of the bacteria. The dead tissues may either break down into pus, which must be evacuated, as in the case of staphylococcal pus, or else when the natural resistance is

good calcium salts are deposited in the dead tissue and the focus becomes calcified. Any condition from a mass of stony hardness, through caseation, to liquid pus may be found, but whatever the condition, the tissue is dead and by no therapeutic means can it be brought to life again. We may assume that if it is freed from bacteria and is only small in size it may be absorbed, or if larger it may be calcified, but these are neither of them processes which can be guaranteed by vaccine therapy. As will be seen later in consideration of the treatment of tubercular glands, some of these disappear entirely under treatment, and it is assumed that these are glands which were merely inflamed and not necrotic : others grow smaller and remain hard and do not vary in size, but still are definitely larger than normal glands, it is assumed that these are glands in which there were small necrotic foci which have been freed from their bacteria and have undergone fibroid or calcareous change. Vaccine therapy cannot be expected to yield better results in such cases than this. Its influence is upon bacteria, it has no direct influence at all upon dead tissues.

In some surface infections, particularly those in the neighbourhood of mucous surfaces, local treatment is often required in addition to vaccine therapy. In such a condition as pyorrhea-alveolaris, there is a mucous surface, namely, the inner aspect of the gum, discharging pus into a septic cavity, the mouth, where there is a large amount of foreign matter with a rough surface, such as tartar, on the teeth. When vaccine therapy is successfully employed, the bacteria lodged in the deep layers of the mucous membrane within reach of the tissue fluids are killed, but there is constant risk of reinfection from the bacteria present in the mouth. The treatment should therefore be combined with most careful and thorough local treatment of the mouth. The same applies to bone disease when a sequestrum is present, or cystitis when a stone is in the

bladder. Even without local treatment considerable improvement often follows inoculation, but it is altogether too much to expect a cure.

In considering the results of vaccine therapy, therefore, it is necessary to bear in mind what results are attainable by that method, and what are not.

But it cannot be said that vaccine therapy is universally successful in every case where it is called upon only to exert an antibacterial effect, and not to perform the impossible in the removal of dead tissues.

It is necessary to inquire into the reasons for these unfavourable results. They may be due to any one of four causes: (i) failure to employ the right vaccine; (ii) failure to employ the right dose; (iii) failure on the part of the body to respond to the stimulus, this response being either totally absent or else being only very slowly developed, or it may be that a positive response is excited, but one which is insufficient to get rid of the infection, and finally (iv) in spite of a successful immunising response there may be failure to determine the blood when enriched with antibodies to the site of the lesion.

The selection of the right vaccine is often a matter of great difficulty, and the lack of success which will attend any error in this direction is a point which need not be further laboured. The question of dose is one only second in importance to that of vaccine. It has to be remembered that the immunising response does not vary directly with the dose. Only doses between certain minima and maxima can be used with good therapeutic effect. Doses below the minimum are too small to stimulate the production of antibodies to a sufficient extent to influence the bacteria in the lesion, while doses above the maximum fix the antibacterial substances already in circulation, and leave the bacteria in the lesion less controlled than before, thus producing the "negative phase."

The minima and maxima within which useful doses may be found have been fairly well determined for the common infections, but occasionally individuals are met with who have strong idiosyncrasies with regard to dose, who may be either unusually susceptible, and suffer a pronounced negative phase from a dose commonly considered minimal, or unusually insusceptible and only able to respond to doses far in excess of the usual maximum. These cases can only be discovered by a careful study of their serum reactions, estimation of the opsonic index being made before and after doses of varying size.

But the source of error against which it is impossible to guard in the light of present knowledge is the total failure to excite an immunising response, which occurs in some cases.

It was claimed in Chapter I that "the animal organism elaborates antibodies to any foreign albuminous substance introduced into it, which is soluble in the tissue fluids," and with regard to healthy animals there is every reason to believe that this is strictly true. But an infected person is not a healthy animal, the presence of bacteria in his lesion is proof of the failure of one part of him to elaborate antibodies efficiently, and it may be that no part, accessible to the vaccine therapist, is capable of responding to the stimulus of the vaccine. This is rarely the case; otherwise vaccine therapy would be useless as a method of treatment; but the success of the method depends entirely on the response of the tissues to a stimulus, and instances occur in which this response cannot be aroused. There are degrees of failure to respond to this stimulus: sometimes it is absolute, sometimes the response is only extremely slow, and improvement may be achieved after prolonged treatment for many months. In other cases a response occurs, with a rise in the opsonic index, but the response is not sufficient to get rid of the invading bacteria. It may easily

be that the resistance of an uninfected person to any organism is such as to be represented by the opsonic index of 1·0, but when that person becomes infected, a serum of the value of 2·0 or 3·0 may be required, containing twice the normal amount of antibacterial substance, to get rid of the bacteria; but his tissues may not respond to inoculation with vaccine to this extent. It may be possible to raise his index to 1·5, but this amount is insufficient, so that though a definite and measurable immunising response is aroused, the lesion may progress and even terminate fatally. Such cases, again, are rare, but they occasionally occur (see chart 11).

A similar case is that in which an immunising response is aroused, with an increase in the opsonic index of the serum, but the antibacterial properties of the blood are not brought to the site of the lesion. This may occur in superficial skin affections and also in deep ones, the latter on account of the presence of stagnant fluids or inflammatory thickening or similar local causes. These cases may be met by appropriate local treatment, by the removal of fluid and dead tissues, and by the determination of tissue fluids to the site of the lesion by hyperæmic methods, the application of heat, massage or passive congestion, and by inducing a flow of fluid blood by the internal administration of citric acid, and in the case of sinuses by the external application of a lotion of sodium chloride and sodium citrate, as described in the chapter on the use of vaccines.

The above are the more likely sources of failure, which require to be taken into account, in any estimate of the usefulness of therapeutic inoculation.

Some idea of the general results attainable by means of vaccine may be gathered from the following summary of cases taken from the Out-patient records of the Department of Therapeutic Inoculation at St. Mary's Hospital.

They are unselected and include every case in which a result is recorded. The pure staphylococcal and the tubercular infections have been chosen as typical instances of acute and chronic infections, and they are also the cases of which the largest records are available. Staphylococcus gives rise to recurrent acute infections.

The results are to be taken for what they are worth. They are compiled from the records of a large department where much practical work is done, and there is small time for elaborate records. The records were not made with a view to publication, and have been in no way edited by the gentlemen who made them. Consequently they are scanty, and doubtless some of them would call for qualification, but they are on the whole the best large and impartial record which is accessible, and it is believed that the present summary gives a fair perspective view of the results attained and attainable by this method under Out-patient conditions.

Staphylococcal Infections.

There are records of 120 cases treated with staphylococcus vaccines only. Mixed infections are not considered. The results are put down in three classes. Class 1 includes those which appear from the records to have been cured. Class 2 includes those which have improved to some extent, but have not been cured. Class 3 includes those which were unchanged or worse.

The great majority of cases are those of abscess in some form or another, and under this name are included cases of boil, furuncle, carbuncle, whitlow and stye.

With "impetigo" are grouped cases of "inflammation," "pus-infection," "rash," "eruption," "eczema," and "pruritus." There are also cases of sycosis, ulceration, and acne, and one of cystitis. The results are tabulated as follows:—

TABLE I.

		1. Cured.	2. Improved.	3. Unchanged.
Abscess . . .	96 cases	67	23	6
Impetigo . .	10 ,,	4	4	2
Sycosis . . .	8 ,,	3	4	1
Ulcer . . .	3 ,,	—	3	—
Acne . . .	2 ,,	—	2	—
Cystitis . . .	1 case	—	1	—
	120 cases	74	37	9

The most important of these groups is clearly abscess, which gives about 70 per cent. cured, 24 per cent. improved, and 6 per cent. failures. But the cure of abscess is not unknown by other methods of treatment, and these figures will not impress anybody much. The question of importance is what results are obtained in persons who are continually suffering from boils, the subjects of recurrent staphylococcal infection.

In the 67 cases of cured abscesses, the time for which the patients had been subject to boils is recorded in 46, and the periods are set out in Table II according as the 46 cured cases had been suffering from abscesses for periods of years, months, weeks or days before treatment was begun.

46 *Cured Cases of Abscess.*

Subject to boils before treatment began for a period of :—

TABLE II.

Years.	Months.	Weeks.	Days.
15 cases.	13 cases.	11 cases.	7 cases.

The majority of these cured cases had therefore been subject to staphylococcal infections for considerable periods.

The next point of importance is the length of time required to effect a cure. Of the fifteen cases who have been subject to infection for years, one took more than a year to cure, seven were treated for months, four for weeks, and three for days.

Of the thirteen cases who had been suffering for months six were treated for months, five for weeks, and two for days. Of those who had been suffering for weeks, one was treated for a year, five for months, four for weeks, and one for days. Of those who came within the first few days of infection, one continued treatment for a year, two for months, two for weeks, and two for days.

Such are the records, but it must be borne in mind that the patients, who pay nothing for their treatment, frequently attend long after all signs of infection have disappeared, with a view to prophylaxis. Viewing these results dispassionately, one may conclude that patients who have suffered from staphylococcal infection for months or years, and who are cured in a few months, have done very well, whereas those who have undergone treatment early in the course of infection and have had to continue for upwards of a year have not immunised well. The records, be it noted, are very rough, and a given date may refer to the duration of a single boil, and not to the period to which the patient has been subject to them.

Tubercular Infections.

Records of results have been obtained in 260 cases of tuberculosis in various sites. The results are estimated slightly differently from the staphylococcal ones, since after chronic tubercular infection, with the consequent destruction of tissue, a cure of the complete kind which may occur after staphylococcal infections is not to be anticipated. As before, the results are grouped in three classes. In Class 1 are included those cases which have been cured or greatly

improved; Class 2 includes those slightly improved, and Class 3 those unchanged or worse. The sites and results are set out in Table III.

TABLE III.

Site of Lesion and No. of Cases.		Class 1. Cured and much better.	Class 2. Slightly Improved.	Class 3. Unchanged or Worse.
Lymphatic Glands .	79	49	18	12
Joints	55	29	20	6
Ulcer Sinus and Abscess	47	30	9	8
Genito-urinary Tracts .	31	15	13	3
Lupus . . .	21	11	9	1
Phthisis	8	4	—	4
Bone	5	5	—	—
Dactylitis	5	2	2	1
Peritonitis . . .	4	1	2	1
Eye	3	3	—	—
Larynx . . .	2	—	—	2
	260	149	73	38

So far as these results are reliable, there are, among 260 cases, 149 greatly improved, 73 slightly improved, and 38 unchanged or worse. Expressed in percentages, neglecting decimals, Class 1 = 57 per cent., Class 2 = 28 per cent., Class 3 = 15 per cent. The most numerous cases are those of tubercular lymphatic glands, of which there are 79 which are classified in the following way:—When glands have disappeared altogether, or have been reduced to a size not larger than cherry-stones, and then remain hard and do not vary in size, they are considered "cured." When there is marked diminution, but the glands remain as large as raisins, they are considered "much better"; these two kinds of results are grouped together in Class 1. When the reduction is slight only the glands are considered

"better," and placed in Class 2, while those "unchanged" and "worse" are placed in Class 3.

The results of treatment are as follows:—Class 1, 49 (27 cured, 22 much better); Class 2, 18; Class 3, 12; or expressed in percentages, Class 1 = 62 per cent., Class 2 = 23 per cent., Class 3 = 15 per cent., and as these are fairly typical of the general results they may be considered in greater detail. The gland-cases were, on the whole, decidedly bad ones: 43, or more than one-half, had been previously operated upon, and 21, or one-quarter, had had more than one operation; five of them had had 10 operations or more. Of the cured cases nine out of 27 had been operated upon.

To consider in some detail the cases which were cured: the period for which the patients had suffered from enlarged glands before treatment began is recorded in 24 cases. Three cases had lasted for more than five years, 12 for periods between one and five years, and seven for less than one year. With regard to treatment of the whole 27, eight were under treatment for more than two years, six for more than one but less than two years, and 13 for less than one year.

A single case may be quoted as a specimen of successful treatment. A man, aged thirty, developed tubercular glands in the neck and an abscess at the point of the shoulder in 1902. In 1903 he was operated on, but the wound did not heal, and five further operations were performed during the year. In December, 1903, the whole skin from the left ear to the left shoulder was ulcerated, with a deep ulcerated crater below the ear, and there was a large gland in the axilla. Inoculation was then undertaken with new tuberculin and a staphylococcus vaccine, and the local application of formalin and gelatin. In eleven months the skin was completely healed except one area the size of a threepenny-piece. Six months later further glands

became enlarged, and broke down; treatment was resumed and the lesions completely healed. From that date to the present time (1910) the patient has kept in very fair health, and though not a strong man is able to be constantly at work.

These are the results obtained in the chronic infection of which there is the largest experience. The literature contains many cases of very brilliant results obtained in desperate cases with vaccine therapy. No such cases have been added to this summary, because they are necessarily individual cases against which the incredulous may bring the criticism of *post hoc, ergo propter hoc.* It is believed that the cases quoted above are sufficient for the formation of an unbiassed opinion on the merits of vaccine therapy as a method of treatment.

CHAPTER VII

THE HISTORY OF THERAPEUTIC INOCULATION,[1] WITH A CRITICAL NOTE ON THE OPSONIC INDEX

In order to keep the argument as free as possible from side issues, no mention has been made in the preceding chapter of the men of science to whose researches the evolution of Therapeutic Inoculation as a method of treatment is due. The following is a brief sketch of the chief steps by which this method has been brought to its present position.

The earliest known attempts to produce immunity by artificial means were directed against small-pox, and were based, presumably, on the observation that after spontaneous recovery patients were protected for life from further attacks of the disease, and further, that they were equally well protected by a mild attack as a severe one. Even at the present time the oldest and still the greatest prophylactic measure in use is vaccination against small-pox, and from this the whole method takes its name. Before this was introduced, prophylactic inoculation against the disease with matter from small-pox pustules was employed in this country for some time. This was a common practice in the East long before it was introduced into England. It was observed in Constantinople by Lady Mary Wortley

[1] Part of this chapter was published in "Science Progress," 1909, under the title of "Vaccine Therapy in Theory and Practice."

Montagu, wife of the British Ambassador, who subjected her own son to it. She introduced the method into England in 1721, in the teeth of bitter opposition, and her grandson, during a severe epidemic, made extensive use of it with brilliant results. He inoculated more than two thousand persons, of whom only three died. Direct inoculation, however, was open to this serious objection—that although the patient himself suffered only from a mild attack, which was followed by immunity, he could none the less convey the infection in a virulent form to others—and the practice was finally prohibited by law.

In 1780 the attention of Edward Jenner was drawn to a vesicular disease of the udders of cows which sometimes infected the hands of dairy workers, who were found to be afterwards immune to small-pox. Instances are recorded of farmers who successfully inoculated their families. Jenner investigated the subject, and in 1796 inoculated a boy with cow-pox and afterwards with small-pox, to which he proved immune. He published his results in 1798, a long controversy followed, and in about 1800 the systematic practice of vaccination began. The results were immediately visible: in London in the two last decades of the eighteenth century the mortality from small-pox was 17,867 and 18,477 respectively, while in the first two decades of the nineteenth it fell to 12,534 and 7,856.

It is remarkable, in view of the wide employment and success of vaccination, that the causal organism of both cow-pox and small-pox is still not certainly determined, so that it is not possible to state definitely whether they are identical or not. It is probable that they are descended from a common origin, and that the organism is modified by passage through a refractory animal. The great principle established by Jenner is that it is possible, by using a modified *materies morbi*, to confer artificial immunity to an infective disease.

A century elapsed from the date of Jenner's first observations before prophylactic inoculation was successfully carried out against any other malady. So long as the infective agents causing disease were unrecognised, experiments were quite dependent upon chance for success, and all recent advance in immunisation has depended upon a knowledge of bacteriology.

The first great step in this direction was taken by Schwann, who demonstrated that organic bodies do not undergo decomposition except in the presence of micro-organisms. This was followed by the work of Pasteur on fermentation, and the identification of the yeast fungus as its cause, in the year 1857.

The probability that disease was caused by similar organisms was by this time suspected; indeed, in 1850 Davaine had observed and described anthrax bacilli in the blood of animals with that disease, though he did not recognise them as the cause. In 1860 Delaford cultivated the bacteria in blood, and in 1863 Davaine showed their constant presence in anthrax blood, and suggested that the disease was due to them. By this time, then, a specific organism had been shown to be constantly present in a certain disease, and to be capable of cultivation outside the body.

It still remained to obtain the organisms in pure culture, which was first done by Koch in 1876, by growing them on a solid medium. This work was confirmed and continued by Pasteur; both these observers produced the disease in animals.

The next problem was how to produce the disease in animals in such a mild form as to ensure recovery, and various modifications in the culture of bacteria have been employed from time to time to "attenuate" the organisms, that is, to preserve their life and identity, but to diminish their virulence. Many efficient methods have been

used for this purpose, and they need not be enumerated here.

In 1881 Pasteur first used a vaccine made from attenuated anthrax cultures, with which he inoculated sheep, and succeeded in rendering them immune. The method of Jenner was thus repeated, employing an organism the relation of which to the disease against which prophylaxis was sought was definitely known. Pasteur called his attenuated culture a "vaccine" in the belief that it stood in the same relation to anthrax as a disease as Jenner's material stands to variola.

In 1885, after many experiments on animals, Pasteur inoculated the first human patient against rabies. In this disease, again, no organism had been isolated, but the virus had been traced to the spinal cords of infected animals, and Pasteur employed emulsions of such cords. The treatment is still in use. Inoculations are used after a person has been bitten by a possibly rabid animal, but only as a prophylactic measure. The incubation period of rabies is a long one—three weeks or more—and it is possible to confer an active immunity before the infective agent reaches the spinal cord. If symptoms have once occurred, treatment is of no avail.

So far no discovery had been made to modify the suggestion of common experience, that immunity could only be artificially conferred by inducing disease in a mild form. But about this period some notable researches widened the outlook very much. Salmon and Smith discovered that animals could be immunised by the products of bacterial growth as well as by cultures of the bacteria themselves. Immunity so conferred was clearly not the result of recovery from the disease.

As far back as 1874 Traube and Gscheidlen had discovered that blood had bactericidal properties. In 1887 Buchner and others confirmed this, and showed that the

properties also belonged to blood-serum, and also that the bactericidal properties of any serum did not extend to all bacteria, only certain ones being affected.

In 1888 Roux and Yersin discovered diphtheria-toxin, a product of the growth of diphtheria bacilli, and Kitasato discovered tetanus-toxin.

In 1890 Behring discovered anti-toxin in the serum of animals immunised against diphtheria-toxin. This was an instance of a specific antibody appearing in the serum as the result of inoculation with a definite albuminous substance.

In 1890 Behring and Kitasato inoculated rabbits with tetanus-toxin, and with their serum conferred immunity to tetanus on mice. In 1891 Behring and Wernicke obtained similar results with diphtheria-toxin and established the law that the serum of an artificially protected animal acquired the power of transmitting immunity to other animals. Herein lies the whole principle of serum therapy. Its importance in treatment, with its limitations, have been pointed out; its importance as a landmark in the study of immunity is of the very first magnitude.

In 1891 Ehrlich, experimenting with the vegetable poisons, ricin and abrin, demonstrated the development of antibodies to these substances and their appearance in the serum of inoculated animals.

In 1894 Calmette demonstrated similar reactions with snake-venom, and in 1894 Bordet produced hæmolytic sera by inoculating animals with the red corpuscles of others of different species. Since that time numerous experiments have been made with many other foreign albuminous substances, and the law of the formation of antibodies has been established beyond question.

These researches entirely changed the conceptions as to the nature of immunity; active and passive immunity were distinguished, and the propriety was recognised of inducing

passive immunity by means of sera in infections with exotoxic bacteria, and active immunity by means of vaccines in endotoxic infections.

The use of vaccines, however, was still confined to prophylaxis, and notable experiments on the human subject in prophylactic inoculation are those of Ferran and also Haffkine with cholera, attenuated living cultures being used for the purpose.

The most important prophylactic measure of recent years is that introduced in 1896 by Wright against typhoid fever. Some large figures are available by which to judge of its efficacy. During the South African War about 19,000 men in South Africa and India were inoculated. Of these 1 in 84 was infected, with a case-mortality of 17 per cent. At the same time, among about 150,000 unprotected men, 1 in 40 took the disease, with a case-mortality of 25 per cent.

This work marks another epoch. It had become clear that active immunity was due to the formation of antibodies, and that recovery was the result and not the cause of immunity. In antityphoid inoculation this knowledge was utilised for the first time in the employment of vaccines made of cultures of typhoid bacilli killed by heat, and experiments made with the serum of the inoculated persons demonstrated that the formation of antibodies was called forth by the dead bacteria.

This modification was adopted after a communication from Pfeiffer that the latter had succeeded in producing an immunising response with a killed culture. Since these experiments only killed vaccines have been used for either prophylactic or curative purposes.

In 1897 Haffkine inoculated himself with a sterilised bouillon culture of the bacillus pestis, and a similar vaccine has since been extensively used in India. Statistics show that some degree of immunity is conferred by the method.

Hitherto, as we have seen, vaccine treatment had been wholly prophylactic, and to Wright belongs the distinction of having first exploited it in a scientific manner as a curative measure. Wright was the first to realise that every organism which causes localised disease, and is capable of pure cultivation, may be employed in the form of a vaccine to cure the disease it causes.

Curative treatment by means of vaccines has been largely modified by the knowledge derived from the study of the antibacterial as distinct from the antitoxic substances of the blood. The main discoveries in this connection may be briefly reviewed. The chief known antibacterial substances are bactericidins, bacteriolysins, agglutinins and opsonins.

As was stated above, the bactericidal properties of blood had been suggested in 1874. In 1886 Fodor made similar observations, in 1887 Buchner followed him, and in 1888 Nuttall demonstrated the bactericidal properties of serum, and showed that those properties were destroyed by moderate heat. In 1894 Pfeiffer showed that a guinea-pig can kill and dissolve a small number of cholera vibrios injected into its peritoneal cavity, and that this power can be much increased by repeated small inoculations. If the serum of an animal thus inoculated is injected into a second guinea-pig the latter will be protected against otherwise fatal doses of cholera vibrios. This, the first evidence of "bacteriolysis," is known as "Pfeiffer's phenomenon."

In 1894 Gruber began to study the agglutination of bacteria by blood serum, and in 1896 Gruber and Widal independently published observations which showed that agglutination of typhoid bacilli by serum may be used as a diagnostic test for typhoid fever.

Opsonins, as has been explained, are bodies in the serum which influence bacteria in such a way as to prepare them for phagocytosis by leucocytes. Phagocytosis is the power

of ingesting foreign bodies which is possessed by amœbæ and also by white blood-corpuscles (leucocytes). The phenomenon was first observed in leucocytes by Haeckel in 1862 in those of a mollusc. Leucocytes can ingest bacteria, and it was long contended by Metchnikoff and his school that immunity depended on this power. The observations of Wright and Douglas show that something further is required.

In 1902 Leishman incubated blood and bacterial emulsions together, and demonstrated varying degrees of phagocytosis with the blood of different individuals.

In 1903 Wright and Douglas, employing an elaboration of this method, demonstrated the opsonic phenomenon described in Chapter IV, namely that leucocytes do not ingest bacteria except in the presence of serum. Phagocytosis was thus shown to depend on some substances in the serum which act on the bacteria so as to render them capable of ingestion by leucocytes. Wright has called these substances "opsonins." If the experiment mentioned is repeated, substituting for the serum of a healthy man that of a person suffering from a local infection by the bacterium in question, it is generally found that his serum has less power of preparing bacteria for ingestion, and that a smaller number of bacteria are taken up by the phagocytes. In practice the average number ingested after using normal serum is regarded as unity, and the number ingested after using the patient's serum is expressed as a fraction or multiple of the normal. This figure Wright has called the "opsonic index."

By this means it is possible to estimate the patient's resistance to the invading organism, and whether benefit is likely to follow the exhibition of a vaccine. The explanation of the low index in local infections is that a focus is to some extent shut out of the blood stream, that the protective bodies have been exhausted in their unsuccessful attempt to destroy the focus, and that bacteria do not

pass into the blood and stimulate the formation of antibodies.

After their early observations that phagocytosis of bacteria by leucocytes did not occur except in the presence of serum, Wright and Douglas proceeded to experiment with different varieties of bacteria, and demonstrated that the opsonic effect was produced on almost all; two only, and those nearly allied, out of ten organisms examined, proved refractory.

The *staphylococcus pyogenes* was then examined in detail, when it was found that persons suffering from pustular infection showed a lower opsonic index to this organism than healthy persons. Further, the pus derived from boils proved to have less opsonic power than the blood. The patients were inoculated with staphylococcus vaccines, and it was found that their opsonic indices rose concurrently with clinical improvement of the lesions.

The tubercle bacillus, the next investigated, presented great technical difficulties, but when these were overcome, the same facts became plain. The indices of patients with localised tubercular disease were found to be lower than those of healthy persons, and the indices of pathological fluids, such as serous effusions, were found to be lower still. Some cases of localised tuberculosis were treated with inoculations of new tuberculin, and considerable improvement was recorded.

In the course of these investigations the possibility of producing a "negative phase" was discovered, namely, the fact that if a large dose of vaccine is given the opsonic index is not raised but depressed, and if an inoculation is given during the negative phase, the index is depressed still further, and the clinical symptoms change for the worse. This is knowledge of the first importance for all who employ vaccine therapy.

If these methods were to be of use in the diagnosis of tubercular disease, it was clearly necessary to find out if

all normal persons offer the same resistance to the tubercle bacillus—that is, if a normal index really exists. Bulloch accordingly investigated the opsonic index of sixty-six healthy persons, regarding himself as normal; he found an average of ·95, the variations ranging from ·8 to 1·2, and concluded that the sera of normal individuals are almost identical in their opsonic content. Very numerous observations on the tuberculo-opsonic index followed these researches; and a fact which was soon elicited was that, while patients with strictly localised tuberculosis have consistently low indices, those who suffer from constitutional disturbance have variable indices, which may be either above or below normal, and it was concluded that such patients live in a constant succession of negative and positive phases, due to the entrance of bacilli into their blood from the focus of infection, and consequent "auto-inoculation."

The possibility of exciting auto-inoculation, which had been long recognised in cases of constitutional disturbance, was first observed in localised disease by Freeman. This observer, while investigating a case of gonococcal arthritis, found one day a marked rise in the patient's index to gonococcus following massage of the affected joint. An extended series of observations followed, which demonstrated that this phenomenon occurs also in bacterial infections after operations, exercise, after deep breathing in the case of lung disease, and after reading aloud in the case of disease of the larynx.

This discovery of the possibility of inducing auto-inoculation in cases of local disease has proved to be of the greatest importance in vaccine therapy for several purposes. The method can be employed as a crucial test in diagnosis, it offers a most valuable means of treatment in certain cases, and it is necessary to bear it in mind in order to devise correct treatment for patients suffering from constitutional disturbance.

These, then, are the important events in the development of vaccine therapy :

1. Jenner's immunisation by inducing a modified infection.

2. The discovery of micro-organisms as the causes of putrefaction, fermentation and disease.

3. The isolation of disease germs in pure culture, and production of the disease by inoculation into animals.

4. Pasteur's immunisation with living vaccines.

5. The discovery of antitoxins in the serum of animals immunised against toxins.

6. The discovery of antibody formation after injection with ricin, venom, blood corpuscles, and foreign albuminous substances generally.

7. The discovery of antibacterial bodies, bacteriolysins, agglutinins, and finally opsonins in serum, after inoculation with bacteria.

8. The discovery that dead bacteria will stimulate the formation of antibodies, and the consequent employment of vaccines of dead bacteria in prophylaxis.

9. The curative use of vaccines of dead bacteria.

10. The employment of the opsonic index in the diagnosis, determination of dose and general management (prevention of auto-inoculation) of bacterial disease, to which may be added the selection of the most suitable site for inoculation.

An account of the history of vaccine therapy is not complete without a word of criticism of the opsonic index. In the preceding pages a good deal has been claimed for it. When first introduced it was received with great enthusiasm as a possible short cut to diagnosis. It was believed that a single observation of the opsonic index would suffice for diagnosis. If the patient were infected he would be "low," and if not, he would be normal, every laboratory set men to estimate opsonic indices, in this belief. Before long,

however, these workers found that they never obtained results twice alike, and apparently came to the conclusions, first that the method was useless in their hands, and secondly that it must therefore be so in anyone else's, and made small allowance for anyone who maintained the contrary. Meantime those who had worked steadily at the subject had made several discoveries, of which the most important was the phenomenon of auto-inoculation. After auto-inoculation the opsonic index of a patient's serum may be either above or below normal, and consequently may also be caught when it is within normal limits, hence the presence of a normal index by itself is no guarantee of freedom from infection.

Apart from this there is no doubt that the accurate estimation of the opsonic index is a matter requiring considerable skill, great care, and very great practice. The method is perfectly useless in the hands of inexperienced persons.

Every step in the process requires knowledge. The preparation of a suitable tubercle emulsion is a matter of an hour's work. Even given properly prepared emulsions and corpuscles, a film imperfectly spread and ill-fixed or ill-stained is a quite useless specimen. But with well prepared films the actual estimation of the index, though wearisome, is not difficult.

Of all the estimations of antibacterial substances in serum the estimation of the opsonic index is the most generally applicable. The opsonic reaction can be demonstrated against nearly all bacteria. A very high degree of accuracy is obtainable in the hands of experienced workers, as has been shown by Fleming in a careful paper published in *The Practitioner* in May, 1908. Every important modification in the use of vaccines has been made in deference to indications given by the opsonic index. It was entirely through this method that auto-inoculation, with its im-

portance in diagnosis and treatment, was discovered, and that dose has been so regulated that a pronounced negative phase is now hardly ever seen. One specific instance of its importance and accuracy may be given. In Wright's laboratory blood samples from two or more workers are used as controls or "normals," and it is found that the indices obtained with these samples are always alike, within narrow limits. On no fewer than three occasions it has happened that the blood of individuals which had at first been normal became abnormal, and in each case a tubercular lesion was afterwards clinically discovered.

One cannot help feeling that positive evidence of this kind is more convincing than any reports of inaccuracy of diagnosis or discrepancy of results, which are from time to time reported by hostile critics.

Although indirect and laborious, and with all its demands on time and technical skill, the estimation of the opsonic index remains the most accessible and the most accurate means of investigating the antibacterial properties of the blood which is available at the present time.

PART II

THE PRACTICE OF THERAPEUTIC INOCULATION

PART II

PRACTICE

CHAPTER VIII

INTRODUCTION

THE following directions for treating some of the commoner infective diseases are based on an analysis of all the cases to the records of which the writer has had personal access, or about which he has received first-hand information, besides those treated by himself. The majority of the statistics are taken from the Out-patient records of the Department of Therapeutic Inoculation at St. Mary's Hospital, and additional use has been made of papers published by workers in that Department on subjects to which they have given particular attention. These are acknowledged in detail in the Preface.

All the records available have been carefully analysed, and in each group of cases the numbers treated, with the results of treatment, the duration of disease before treatment began, the length of the period of treatment, and any circumstances which have had influence on the case, are set down, so that the reader may have an opportunity of forming some individual judgment of the value of the treatment.

The results are classified under the following heads:—1. Cured. 2. Much better. 3. Better. 4. Unchanged. 5. Worse. The good results, those "cured" and "much better," are considered in greater detail, and from these some general directions are deduced for the treatment of similar cases.

It has been thought best to set forth in a dogmatic manner the following definitions and general directions for treatment.

Definition of a vaccine.—A vaccine is a sterilised suspension of bacteria of known numerical strength. The fluid used to suspend the organisms is 1 per cent. sodium chloride solution containing $\frac{1}{2}$ per cent. carbolic acid.

Unit of volume.—The accepted unit of volume is one cubic centimetre. No fixed standard of strength is possible, since vaccines of different strengths are required for various purposes; it is therefore necessary for every vaccine to be labelled as containing so many millions of organisms per cubic centimetre.

Dose.—The dose is estimated in millions of bacteria (with the exception of tubercle vaccine), so the volume of any dose has to be calculated from the strength of the individual vaccine to be used. Tubercle vaccines are made of comminuted organisms and are estimated by weight, each cubic centimetre containing a certain fraction of a milligramme. The tubercle vaccine referred to in the cases quoted is Koch's "bacillary emulsion."

Storage.—Vaccines are stored either in glass capsules or rubber-covered bottles; for private use generally in the former.

Withdrawal.—The vaccine is withdrawn from the capsule thus:—The thin end of the capsule is broken off and the capsule is inverted. The needle attached to the nozzle of a sterile hypodermic syringe is now introduced

into the capsule and the required amount of vaccine is withdrawn (see Plate 2). The capsule may now be sealed by fusing the open end in a flame and reserved for further use.

If the vaccine is stored in bottles, it may be withdrawn by inverting the bottle, thrusting the needle through the rubber cap and withdrawing the amount required. The cap is smeared with lysol before withdrawal. If a fine needle is used the rubber will close up after the puncture and the bottle will remain air-tight.

Site of inoculation.—Vaccines are intended for subcutaneous injection. Any part well covered with fat may be selected for injection of the vaccines which do not produce much local inflammation. The back of the humerus is a convenient site for male patients, but for female patients the back of the shoulder over the supraspinous fossa is generally more accessible or the skin of the thigh a little way above the knee. For patients in bed, or when vaccines are used which produce much local reaction, convenient sites are the abdomen an inch internal to the iliac crest, or the thorax an inch below the clavicle. In both these sites there is plenty of loose tissue under a bony arch where any swelling which may occur will be protected from pressure.

Preparation of the skin.—If the skin is clean no special preparation is required. The site selected for inoculation should be smeared with pure lysol immediately before injection, and this must be wiped off immediately afterwards or the skin will be burnt. Only a sufficient area to allow of the insertion of the needle need be thus prepared: a space $\frac{1}{8}$th of an inch in diameter is sufficient.

For inoculation purposes the finest possible needle is desirable, and it must be kept thoroughly sharp. The site for inoculation should be gently pinched up between the

fingers of the left hand and the needle thrust in quickly, very little pain will then be felt. The object of this is to afford the greatest resistance to the needle point at once. If a yielding site is chosen the insertion of the needle will be more painful.

Sterilisation of needles and syringes.—This is conveniently done with olive oil which is heated in a small metal bowl standing on a tripod over a gas burner or spirit lamp. If it is required for constant use it is advantageous to use gas controlled by a thermo-regulator in the oil, but if the oil is once heated to the required temperature of 140° C. and the by-pass of a Bunsen burner is kept alight under it, the oil will keep hot and may be brought to the full temperature in a few seconds by turning up the gas. The oil should not be allowed to get above 150° C. or it will begin to smoke and smell. Needles and syringes may be sterilised by simply filling with oil at 140°. Good glass will bear this temperature, but the risk of cracking is reduced if the syringe is half-filled with air before any oil is drawn into it. The syringe, when half-filled with oil, should be rolled round in the hands so as to heat its whole surface. After this it may be completely filled in the ordinary way. The best syringe for the purpose is that known as the "Record."

Blood cultures.—The principal clinical details of the administration of vaccines are included above, and the technique of drawing blood for bacteriological culture may be conveniently described in this place. For the purpose of culture, blood is drawn from a vein. Any vein large enough to admit a needle may be chosen. The most convenient is generally the median basilic. The procedure is as follows:—The skin over the vein is scrubbed with soap and water and covered with a sterile swab. The arm is congested by a rubber or linen bandage which should be

finished with a bow which can be easily undone. The patient is then put in a sitting or lying position and the arm is allowed to rest on a table or some such support. A bandage or folded handkerchief is placed in the patient's hand, and he is directed to close his fist firmly upon this. The veins will now stand out clearly. Immediately before drawing off the blood the site selected is touched with pure lysol which is washed off at once with absolute alcohol. A 10 c.c. syringe is required: this must have been previously sterilised with oil, which may be washed out by filling the syringe with sterile sodium carbonate solution, 1 in 500: then about half a cubic centimetre of sterile 1·5 per cent. sodium citrate solution is taken in the syringe and all is ready for drawing the blood. A needle is required of fairly wide bore, but it need not be as large as that required for the aspiration of pus. A really sharp needle is indispensable to a successful blood culture. The selected vein is held steady by the left hand, one finger being placed on each side of it. The needle of the syringe is held at an angle of 45° to the vein, it is thrust quickly through the skin and into the vein for a distance of about $\frac{1}{8}$th of an inch. This should carry it into the vein. The point of the needle is now turned slightly upwards in the direction of the vein. The barrel is now taken in the left hand and the piston withdrawn with the right slowly and steadily. If the needle is in the vein, blood will immediately flow into the syringe; if not, it will sometimes flow if withdrawn for a little distance, failing this the vein must be felt for with the point of the needle again. When blood begins to flow a steady pull is maintained on the piston till the syringe is quite full. This should take about 10 seconds. When full the syringe is again taken in the right hand without moving the point of the needle, a sterilised swab is placed over the point where the needle was inserted and the syringe with-

drawn with the right hand. If there is no assistant the syringe may be put down for a moment and the bandage released with the right hand. If this is done quickly there will be no bleeding from the puncture. Six broth tubes must be ready to receive the blood and the 10 c.c. of blood should be divided among them.

CHAPTER IX

DISEASES OF THE SKIN

The following is an analysis of the records of 190 cases of various forms of infection of the skin, together with 50 cases of erysipelas described by Ross and Johnson of Toronto. They are grouped under various heads. The principal classification is into (I.) Non-tubercular and (II.) Tubercular infections, each of which includes smaller groups.

(I.) *Non-tubercular cases.*—These include the following groups:

1. *Abscess*, which includes focal subcutaneous lesions, chiefly staphylococcal, variously described as "abscess," "boil," "carbuncle," "furuncle" and "stye." Cases of whitlow are considered in the same group.
2. *Ulcer*, including cases described as "rash," "scab," "impetigo," "infected wound," "pus infection," "ulcer," "eruption," "eczema" and "pruritus."
3. Acne, sycosis, and folliculitis.
4. Erysipelas.

(II.) *Tubercular cases* are divided into two large groups:

1. Lupus.
2. Tubercular ulceration, including cases of ulcer, sinus, and abscess.

(I.) *Non-Tubercular Lesions.*

1. *Abscess.*

The total number of records analysed is 104, and the results of treatment have been as follows:—

Cured	73
Much better	15
Better	14
Unchanged	1
Worse	1

Relapse has been recorded in 12 instances, and in 4 transient but definite ill-effects of various kinds have followed inoculation.

To consider more closely the 73 cases that have been cured:

Age.—The age incidence shows nothing remarkable. The cases have been rare under the age of 10 and rare over 40, but from 10 to 40 they are quite evenly distributed:—from 10 to 20, 24 cases; from 20 to 30, 29 cases; from 30 to 40, 24 cases. About the same proportion of cases are cured in each age group considered. Up to 10 years old there are 57 per cent. cured; between 10 and 20, 64 per cent.; between 20 and 30, 82 per cent.; between 30 and 40, 70 per cent.; above 40 years about 60 per cent.; so that age has little bearing on the prognosis.

Organism.—The organism found has been staphylococcus only in 67 out of the 73 cases; in 1 it has been streptococcus only, and in 5 a mixture of staphylococcus and streptococcus. This group is therefore practically one of staphylococcus infection.

Dose.—The dose is considered in minima and maxima. Minimal doses practically correspond to initial doses. Those given in this series are shown in the table below.

Doses.

Minima.		*Maxima.*	
50 millions	in 16 cases	100 millions	in 1 case
75 ,,	4 ,,	150 ,,	7 cases
100 ,,	27 ,,	200 ,,	3 ,,
150 ,,	11 ,,	250 ,,	15 ,,
200 ,,	8 ,,	300 ,,	9 ,,
250 ,,	8 ,,	350 ,,	10 ,,
350 ,,	1 case	500 ,,	23 ,,
		and more than 500 ,,	1 case

Interval.—Inoculations are given at intervals of from three days to a week.

Duration of the disease before case came under treatment and duration of treatment.—In the cured cases of the series this period has been recorded in 52 instances. The duration has been a period of days, that is, less than 2 weeks in 10 cases; a period of weeks, that is, less than 2 months in 13; a period of months, less than 1 year, in 13 cases; and a period of more than a year in 16 cases.

These periods generally indicate the time to which people have been subject to boils and not to individual lesions.

Of the cases described as cured, 11 remained under treatment for a period of days, 31 for a period of weeks, 28 for a period of months and three for a period of years.

A note is required on those cases in which patients are suffering from an abscess which contains pus, but is too deeply seated to point, although no clear records of such appear in this series. It is a question for consideration whether it is wise to attempt to abort such an abscess by the inoculation of small doses, thus attempting to raise the patient's resistance, or to attempt to induce a negative phase by a large dose and so encourage the abscess to break down and point, and so to be got rid of. It is a matter which must be decided on clinical indications. If

there is much pus present it will be absorbed only very slowly if at all, and a painful lesion will be present for a considerable period. The attempt to induce breaking down is frequently carried out with success; the patient receives a dose of 500 millions at the outset.

From these results some generalisations may be made. The diagnosis of the infected organism may be made on clinical grounds. A stock vaccine is nearly always efficient. Except in the cases of deep-seated abscess just mentioned, a dose of 50 millions should be given at the outset and the patient should be seen again in three days. The next step must be decided on clinical grounds. As a rule there is some degree of improvement by this time, in which case 100 millions may be given and repeated in a week or at the end of this time increased to 200 millions, and if the lesions do not clear up, the dose may be gradually increased to 500 millions, an amount which it is rarely necessary to exceed.

Staphylococcus vaccine is one to which the susceptibility of individuals varies considerably. The point to be observed is the degree of reaction which follows each dose. If there was some degree of improvement without an increase in symptoms, the dose may be increased by 100 millions at intervals of from one to two weeks. If the improvement has been considerable after any given injection there is no occasion to increase it. If there has been an increase in symptoms of any severity, the dose should be reduced to that which did not give rise to such symptoms.

Case of whitlow.—Seven cases have been treated, six of which have been cured and one unchanged. Twice additional treatment by incision has been adopted. The age incidence is of no importance, as the cases have occurred indifferently between the age of 10 and 40. Each case has been treated with staphylococcus, and three have

had an additional streptococcus infection. The dose of staphylococcus has as a rule been small; three of the cured cases have had a minimum dose of 50 millions, and three have had a maximum dose of 100 millions, only one instance is recorded of a dose of 400 millions. If a streptococcus vaccine has been used, the doses have varied between 5 and 10 millions. The duration of the infections has not been recorded. As to the duration of treatment, three have been treated for days, two for weeks and one for months. The general rules of frequency of dosage are the same as for ordinary cases of abscess.

2. *Ulcer, etc.*

Records of 39 cases have been analysed. The general results of treatment have been as follows :—

Cured	17
Much better	10
Better	10
Unchanged	2

Relapse has been recorded in three cases.

The duration of the disease before treatment began has been recorded in 24 cases as follows :—For days, two cases; for weeks, six; for months, seven; and for years, nine.

The age of the patients.—Up to the age of 10, four cases, two cured or much better; from 10 to 20, eight cases, six much better; from 20 to 30, six cases, all much better; from 30 to 40, six cases, all much better; and above 40, eight cases, seven much better.

The site of the lesion.—Face and head affected in 16 cases; neck in two; upper extremities in nine; lower extremities in seven; body in three.

Organism.—Pure staphylococcus was found in 16 cases, and pure streptococcus in five cases. Staphylococcus and streptococcus were combined in 18 cases.

Dose.—The doses of staphylococcus vaccine have been as follows:—

Minima.		*Maxima.*	
50 millions in	5 cases	50 millions in	1 case
100 ,,	14 ,,	100 ,,	2 cases
150 ,,	4 ,,	150 ,,	1 case
200 ,,	3 ,,	200 ,,	3 cases
250 ,,	4 ,,	250 ,,	10 ,,
300 ,,	1 case	300 ,,	4 ,,
		500 ,,	6 ,,
		more than 500 ,,	5 ,,

The doses of streptococcus vaccine have been as follows:—

Minima.		*Maxima.*	
Half a million in	1 case	2 millions in	1 case
1 million in	1 ,,	3 ,,	1 ,,
1½ millions in	1 ,,	4 ,,	1 ,,
2 ,,	1 ,,	5 ,,	5 cases
2½ ,,	2 cases	7 ,,	1 case
3 ,,	6 ,,	7½ ,,	3 cases
4 ,,	1 case	8 ,,	1 case
5 ,,	8 cases	10 ,,	7 cases
7 ,,	1 case	12 ,,	1 case
10 ,,	5 cases	15 ,,	5 cases

The cases were under treatment for the following periods:—

Weeks	13 cases
Months	13 ,,
Over one year . .	1 case

The following points are also noted: a sequestrum was removed in one case; special local treatment by means of *X*-rays and other methods is recorded in one case, and a special treatment of syphilis is recorded in one case.

From these figures the following general rules may be deduced:—With any case of rash, ulceration and sinus not due to the tubercle bacillus, the infection is due in the majority of cases to either the staphylococcus or strepto-

coccus, or more frequently both together. The diagnosis should be made on the results of the examination of films and cultures from the lesions. The safe treatment is to use 100 millions of staphylococcus and 3 millions of streptococcus as the initial dose which may be repeated at weekly intervals and gradually increased, increasing the staphylococcus vaccine by 50 millions at a time and the streptococcus by 1 million. In the majority of cases 250 millions of staphylococcus is a sufficient dose, but that may be increased quite safely in simple cases to 500 millions. The average maximum dose of streptococcus is 10 millions, but this may be increased to 15 millions as occasion requires. Treatment is of long duration, and in the majority of instances at least three months is required.

3. *Acne, sycosis and folliculitis.*

The pathology of acne vulgaris, and its treatment by vaccines, has been studied by Alexander Fleming (*Lancet*, April 10th, 1909), and from his paper the following account is abstracted :—

A bacillus was described in the lesions of acne vulgaris by Unna in 1893, it was cultivated by Sabouraud in 1897 and redescribed by Gilchrist in 1899 and 1903.

Morphology. The bacillus varies in length from 1 to 4μ and is about $\frac{1}{2}\mu$ in width. It stains by Gram's method, but irregularly, and is decolourised without much difficulty. It occurs both singly and in pairs, the latter often arranged like a V, and also in irregular groups ; it probably belongs to the diphtheroid group. It is frequently to be found in smears from the pus. In a series of more than 100 cases the acne bacillus was found alone in 44 per cent. and associated with staphylococcus in 53 per cent.

Culture. The bacillus is very difficult to cultivate. It can be grown in broth, on acid agar and glycerine agar. The best medium is one devised by Fleming which contains

·1 per cent. of oleic acid. This was suggested by the frequent presence of the organism in oily secretions. It grows best anaërobically.

Serum reactions. Agglutination can sometimes be obtained with the serum of infected persons, not with that of healthy persons. The opsonic index is variable. Infected persons commonly have a higher index than healthy persons.

The bacillus is pathogenic for mice, and if rubbed into the skin of a susceptible person it may be followed by a pustular folliculitis. Fleming once produced this reaction. In healthy persons no result is obtained.

Authorities differ as to the pus-forming properties of this organism. Fleming's reasons for including it with the pyogenic bacteria are, among others, the following:—The bacillus is constantly present in the pus, it is often the only organism present and may be obtained in pure culture; rubbed into the skin it may produce pustular folliculitis. An overdose of vaccine leads to pustulation, correct dose leads to improvement in the pustules. The opsonic index is high in persons who are improving, and when the opsonic index is low, fresh pustules are formed.

Treatment by vaccines. Patients may be divided into three groups:—(1) Those suffering from comedo and acne pustules only; (2) Those infected with both acne and staphylococcus; (3) Those who are infected with staphylococcus only, the lesions of which approximate to the furuncular type.

Diagnosis may be made by microscopic examination of pus.

Fleming quotes illustrative cases and concludes that the dose should be from 4 to 10 millions at weekly intervals. A stock vaccine may generally be used, but a special vaccine is sometimes required. The treatment should be regulated by clinical indications.

Records of 67 cases have been analysed by the present writer.

Age. Up to 10 there were two cases. From 10 to 20, eleven cases; 20 to 30, twelve cases; 30 to 40, four cases; and over 40, four cases.

Results. Of these

12 are cured,
21 are improved,
21 slightly improved,
12 unchanged, and
2 worse.

Relapse has occurred in six cases.

Duration. Of the cases cured and much better, three had suffered for a period of weeks, two for a period of months, and 20 for a period of years. They have been treated with staphylococcus and with acne vaccines.

DOSE.

Staphylococcus Vaccine.

Minima.			*Maxima.*		
50	millions in	3 cases	150	millions in	1 case
100	,,	12 ,,	200	,,	1 ,,
150	,,	3 ,,	250	,,	10 cases
200	,,	5 ,,	300	,,	2 ,,
250	,,	3 ,,	350	,,	2 ,,
more than 250	,,	2 ,,	450	,,	4 ,,
			more than 500	,,	8 ,,

Acne Vaccine.

Minima.			*Maxima.*		
2	millions in	4 cases	5	millions in	2 cases
4	,,	11 ,,	6	,,	2 ,,
5	,,	5 ,,	7	,,	1 case
6	,,	2 ,,	8	,,	3 cases
8	,,	3 ,,	10	,,	10 ,,
10	,,	3 ,,	15	,,	3 ,,
			20	,,	2 ,,
			40	,,	2 ,,
			50	,,	2 ,,
			more than 50	,,	1 case

Duration of treatment. Six cases for periods of weeks; 18 for periods of months; nine cases for periods of years.

Bad results following excessive doses have been noted in two cases; this is probably an under-statement.

Treatment. Treatment should be conducted on the following lines:—Diagnosis should be made by examination of the pus, and according to that indication doses of 100 millions of staphylococcus and 4 millions of acne given at weekly intervals, being raised according to clinical indications by 50 millions of staphylococcus and 1 million acne till a total of 250 staphylococcus and 8 or 10 millions acne is arrived at. Treatment will probably have to be conducted for about a year, and may have to be resumed at intervals later.

4. *Erysipelas.*

George W. Ross and W. J. Johnson, of Toronto, have studied the treatment of erysipelas with a specific vaccine (*Journal of the American Medical Association*, March 6th, 1909).

Erysipelas is due to the *streptococcus erysipelatis* of Fehleisen. It is an infection of the lymph spaces of the skin, with or without involvement of the cellular tissues. The local symptoms are due to multiplication of the bacteria, and the general symptoms, malaise and high temperature, to toxæmia rather than septicæmia, organisms being but rarely obtained from blood culture. Treatment should be directed towards killing the organisms.

The authors report upon 50 cases, in the first 16 of which the opsonic index was estimated. In two of these, which were on the way to recovery, the index was about normal; in 14 it was below normal. One of these patients died, all the rest recovered, and with recovery the opsonic index rose. The remaining 34 cases were treated without reference to the index. Of the 50 cases, 18 are described as very severe, 20 less severe, and 12 as mild. Inoculation

was followed by a striking improvement in the symptoms of toxæmia, such as unrest, delirium, and malaise. Locally, there was spread of the lesion until after the second and sometimes the third inoculation, but although larger, the lesion showed less redness, swelling, tenderness and pain.

For purposes of comparison they have tabulated 19 cases, being all the cases treated without vaccines at the General Hospital, Toronto, during the year 1907, together with 19 cases treated with the specific vaccine during the year 1908, as follows :—

Method of Treatment.	Pyrexia completed in 24 hours.	Pyrexia completed in 24 hours.	Average duration of Pyrexia.	Complications.	Average duration of Illness.	Average stay in Hospital.
Non-Specific— Series 1907 : 19 cases	3	13	8·9	6	25·0	18·0
Specific Vaccine— Series 1908 : 19 cases	7	5	3·1	1	12·8	11·2

In addition to the results appearing in this Table, they found among the untreated cases several instances of very long duration, some up to 30 days, and various severe complications. Both these were absent in the cases treated by vaccines, except for a single case of abscess in the face.

Treatment. Stock vaccines are used which are made from organisms derived from a large number of cases. In severe cases, 10 millions are inoculated at the first visit; in mild cases 20 millions are given. On the second day, 5 or 10 millions are given according to the severity; after this on alternate days, 5, 10, or 20 millions are given until the temperature has been normal for a week. They are guided as to dose entirely by clinical symptoms; the more severe the condition, the smaller the dose.

They select a site for inoculation far removed from the lesion.

II. *Tubercular Diseases of the Skin.*

These are considered under two heads :—

1. Lupus,
2. Lesions of the skin collected under the titles of "ulcer," "sinus," and "abscess."

1. *Lupus.*

Twenty-one cases were treated. The results of treatment have been as follows :—

Cured	3
Much better . . .	9
Better . . .	8
Unchanged . . .	1

Relapse has occurred in five cases.

Where ages have been recorded they are as follows :— Up to 10 years of age, two cases ; from 10 to 20, six cases ; 20 to 30, seven cases ; 30 to 40, three cases ; and above 40, three cases.

The duration of the disease before treatment has been recorded in 16 cases ; one case was of a month's duration and 15 had lasted for years.

DOSES.[1]

Minima.

1/20,000th	of a milligramme	in 3 cases
1/15,000th	,,	4 ,,
1/10,000th	,,	4 ,,
1/8,000th	,,	1 case
1/6,000th	,,	3 cases
more than 1/6,000th	,,	2 ,,

Maxima.

1/12,000th	of a milligramme	in 2 cases
1/6,000th	,,	1 case
1/5,000th	,,	1 ,,
1/4,000th	,,	11 cases
1/3,000th	,,	1 case

[1] The tubercle vaccine used was Koch's "bacillary emulsion."

It will be seen that the doses used in this disease are large.

Other organisms, chiefly staphylococcus, streptococcus and diphtheroid bacilli were present in 14 cases.

Period of treatment. Eight cases were under treatment for months and ten for more than a year.

Treatment. In the treatment of lupus, therefore, a fair degree of improvement may be anticipated, but cure is rare. The initial dose should be 1/15,000th of a milligramme, and this may be pushed up rapidly to 1/4,000th. Treatment should be given at intervals of a week or ten days, and it will have to be continued for at least a year. Examination must be carefully and frequently made for infection with other organisms; when present these may be treated with the doses given under Abscess (non-tubercular). It is frequently found in practice that the treatment of lupus is complicated by the presence of syphilis, and when this is the case, good results will not be obtained unless specific remedies are employed.

2. *Cases of tubercular ulcer, sinus and abscess.*

Fifty-two cases were treated, and the results were as follows:—

Cured	10
Much better	19
Better	10
Unchanged	9
Doubtful	4

Relapse occurred in five cases.

Age. The vast majority of patients have been under 30 years of age. So far as the ages have been recorded, they were as follows:—Under 10 years, seventeen cases; from 10 to 20, fourteen cases; 20 to 30, twelve cases; and over 30 years of age, five cases. Most of the cases have been chronic. Eleven had been in progress for a period of weeks, twelve for a period of months, and twenty-one for a period of years.

Doses.

Minima.

1/30,000th	of a milligramme	in 2 cases
1/25,000th	,,	4 ,,
1/20,000th	,,	6 ,,
1/15,000th	,,	5 ,,
1/12,000th	,,	1 case
1/10,000th	,,	6 cases
1/8,000th	,,	4 ,,
1/6,000th	,,	3 ,,
more than 1/6,000th	,,	2 ,,

Maxima.

1/20,000th	of a milligramme	in 1 case
1/15,000th	,,	3 cases
1/12,000th	,,	1 case
1/10,000th	,,	2 cases
1/8,000th	,,	9 ,,
1/6,000th	,,	3 ,,
1/5,000th	,,	6 ,,
1/4,000th	,,	9 ,,
1/3,000th	,,	4 ,,
1/2,000th	,,	1 case

Secondary infections by the staphylococcus, streptococcus or diphtheroid bacillus were treated in 30 cases. Treatment had to be long continued; 25 cases were under treatment for a period of months and 26 for a period exceeding a year.

In the treatment of tubercular ulceration the initial dose may be 1/20,000th of a milligramme, and this may be pushed up fairly rapidly to 1/10,000th and increased gradually thereafter. It is rarely necessary to exceed 1/4,000th of a milligramme. Secondary infections are extremely common and in the majority of cases require treatment. The actual lesions themselves must be treated according to ordinary surgical methods. There are many instances, particularly in sinuses, in which there is much coagulated lymph which does not allow of transudation and in which organisms frequently lodge. In such cases great benefit often follows an increased transudation of lymph. This may be induced by the use of a lotion con-

taining 5 per cent. sodium chloride and ·5 per cent. of sodium citrate. The strong salt solution attracts fluid from the tissues, and the citrate, combining with the calcium salts of the lymph, delays coagulation so that the flow of lymph through the lesion is increased.

CHAPTER X

DISEASES OF THE ALIMENTARY TRACT

The diseases of the alimentary tract which have been subjected to vaccine treatment up to the present time to any considerable extent are not numerous. They are practically confined to three, namely:

(1) Septic conditions of the mouth, of which the best understood is pyorrhœa alveolaris;

(2) The group of diseases collected under the term "colitis," and

(3) Diseases of the peritoneum.

Individual cases of other diseases have been treated occasionally, but not in sufficient numbers to allow of generalisation.

Pyorrhæa alveolaris.

When the pus from the alveolus in cases of this infection is examined, a very large number of bacteria is invariably found. Specimens of streptothrix, yeast, coccus, bacillus, spirillum, comma, fusiformis, may be present in immense numbers and in great variety, and it is quite impossible to say that any one organism is the prevailing one present. But of all these bacteria very few are capable of cultivation on ordinary media; only some three or four varieties are commonly obtained, often not so many. A streptococcus,

however, is nearly invariably present: it forms short chains generally of not more than eight individuals and frequently less. Other bacteria commonly cultivated are staphylococcus, pneumococcus, micrococcus catarrhalis, and the pseudo-diphtheria bacillus; the two latter names probably include many different organisms. It is clearly a matter of very good fortune if one of these few which it is possible to cultivate is the infecting organism of so large a host.

With regard to the *streptococcus brevis;* some eminent bacteriologists have thrown doubt on the pathogenicity of this organism. It was investigated with many other streptococci by Washbourn, who concluded from his experiments that it was not pathogenic, and consequently many workers on the hygiene of the mouth have not thought it necessary to consider it. The present writer is of the opinion that this organism should not be so neglected. He proceeds on the following hypothesis.

The *streptococcus brevis* is a common parasite of the mouth, and in the majority of cases it is not pathogenic. It may, however, become so for some reason that is not understood, in the same way as the *bacillus coli communis* is generally a harmless parasite in the large intestine, but may become pathogenic without known cause. He believes that the short streptococcus is in the majority of cases the original cause of pyorrhœa alveolaris, and that infection by other organisms is frequently superimposed.

A point which requires emphasis in the treatment of this condition is that the local treatment by a skilled dentist is essential to success. The mouth is a septic cavity, and it is quite unreasonable to expect that an increase of antibodies in the blood can clear that cavity of organisms. The general hygiene of the mouth must be attended to by local antiseptics, special attention being given to the pockets between the teeth and gum where the

pus collects. Much good may be done by local treatment alone, but in the deep layers of the mucous membrane are organisms which antiseptics cannot reach, and the cure of the condition by local treatment is notoriously difficult. These bacteria are, however, accessible to antibodies in the circulating lymph. Hence the use of both local measures and vaccine therapy are required for the successful treatment of the condition.

The records of twenty cases of simple pyorrhœa without complications have been examined; and of six other cases treated on account of lesions in other sites possibly due to infection in the mouth.

Simple Pyorrhœa Alveolaris. The cases have, on the whole, been mild ones, and with few exceptions have undergone systematic local treatment at the hands of a dentist. All the cases have been treated by vaccines made from the short streptococcus of the mouth and the doses have varied from 10 to 50 millions. This dose is considerably larger than is used in most of the other streptococcal infections. The usual initial dose is 20 millions, which may be increased to 30; it is rarely necessary to exceed this. The duration of the disease before treatment began has, in the majority of cases, been considerable; usually several years, in one at least as many as twenty.

Treatment has usually lasted from one to two months.

Results have been as follows:—

Cured	6 cases
Much better	7 ,,
Better	4 ,,
Unchanged	3 ,,

Secondary infections have been treated in seven instances: four times with micrococcus catarrhalis, once with a diphtheroid bacillus, and once with a coliform bacillus. The doses of vaccines of these secondary organisms have all been from 10 to 30 millions.

A few of these cases require a special note. One was a case rather of acute gingivitis than pyorrhœa. There was a copious discharge of saliva with a great deal of pus and much fetor. This case was much improved after the first dose and cured completely in about three weeks. Another case had suffered from pyorrhœa for 20 years: several teeth had been lost. Treatment was carried out for two months, in which time the condition improved, many of the teeth tightened appreciably and the patient was able to eat food of a more solid character than had been possible for many years. This improved condition persisted for about a year, at the end of which time there was a relapse which was treated successfully by the same methods, but a more severe relapse occurred in a few months and it was decided to remove the teeth. A third case suffered from an inflammatory condition of the papillæ of the tongue, which at once improved with vaccine treatment. In a fourth there was marked improvement in digestion. Relapse has only been recorded in two instances.

There remain six cases of lesions in distant organs which have been thought to be due in the first instance to the pyorrhœa, much attention having been paid of late to oral sepsis as a cause of disease in other parts. The present small series appears to have a little bearing on the view that arthritis and similar lesions are very frequently due to this condition. Four of the six were cases of arthritis and two of choroiditis. The cases of arthritis were all severe and chronic, and associated with varying degrees of oral sepsis.

In one of these cases the oral sepsis was of a serious character; the *streptococcus brevis* was obtained from the pus, and a very marked improvement indeed followed the use of the corresponding vaccine. Local treatment for the arthritis by massage and electricity was also adopted and was partly responsible for the good result.

In the other instances the oral sepsis has, as a rule, not been of a grave character, and in only one of them has any improvement ensued on treatment with vaccines derived from the organisms present. It appears to be open to doubt if mild forms of oral sepsis such as are extremely prevalent among otherwise healthy persons are likely to give rise to grave infections in distant organs.

Of two cases of choroiditis, one improved considerably on vaccine therapy, and one was apparently unaffected.

Colitis.

The following observations are taken from a communication made by Dr. John Matthews to the Medical Society in the course of a Discussion on the treatment of colitis. The writer is indebted to Dr. Matthews for the details, as they have not yet been published elsewhere. He has investigated the relation of the coliform bacilli of the fæces to the condition, and his conclusions, which are based on the study of about 40 cases, including two of acute ulcerative colitis, are as follows :—

Diagnosis. This is a matter of extreme difficulty, and very elaborate proceedings are necessary to detect the infecting organism ; the most numerous is not necessarily the offender. The opsonic index has been relied upon as a means of diagnosis, according to the following process :— A specimen of fæces is obtained and a thin emulsion is made, which is planted out upon plates of neutral-red-bile-salt-agar (MacConkey's medium), which has the property of inhibiting the growth of streptococci, while that of coliform organisms is not interfered with. Upon this medium some red and some white colonies are commonly obtained which form a rough means of division into organisms which ferment lactose and others which do not. As many different coliform bacilli as possible are isolated, and separately planted in a series of sugars in peptone broth to determine

their fermentation reactions. The opsonic index is taken to each of those separated, and it is frequently found that whereas the patient's serum gives a normal index to three out of four organisms, to one it gives a widely divergent index. This is considered the infecting organism. The *bacillus acidii lactici* (Hueppe) has been the commonest in this series; others have been the *B. lactis aerogenes* and *B. coli*, and, rarely, the paratyphoid, paracolon and dysentery bacilli.

Results. Nearly all cases have been in women. Only three have been complete failures. Young subjects are much more successfully treated than middle-aged. It is difficult to be dogmatic about the results, but the majority of patients are sufficiently satisfied to persist in treatment for many months. It is remarkable that in only one of these cases has the bladder been infected.

Dose. This is extremely variable. In some a million is frequently an efficient dose as judged by its effect on the opsonic index; others have gone up to the enormous dose of 5,000 millions without reaction. It will be seen therefore that no dogmatic statement can be made about treatment in this condition. Each case must be judged on its own merits and none is likely to be successful without considerable patience. So far as they go these results appear to be satisfactory in the treatment of a chronic and difficult condition.

Diseases of the Peritoneum. Tubercular Peritonitis.

Six cases. *Age.* Above 10 years of age, three cases; between 10 and 20 years of age, one case; between 20 and 30, one case; between 30 and 40, one case.

Results. None of them are described as cured, but three are much better, one is free from pain and has been discharged from treatment; the other three have improved slightly. In all cases the infection has been with the

tubercle bacillus, and in one there has been an additional infection with staphylococcus.

The doses have been on the whole large. The smallest initial dose was 1/25,000th of a milligramme and in two cases a maximum of 1/6,000th had been given.

In tubercular disease of the peritoneum, therefore, some improvement may be anticipated from the use of tuberculin. Treatment has been long in every case, the usual period being six months.

CHAPTER XI

DISEASES OF THE RESPIRATORY TRACT

RECORDS of the following diseases of the respiratory tract, treated by vaccine therapy, have been examined:

1. Phthisis.
2. Pleurisy and empyema.
3. Whooping-cough.
4. Bronchial asthma.
5. Chronic bronchitis.
6. Nasal discharge.
7. Pneumonia.

Phthisis. Twenty-six cases in all have been treated, including two cases of tubercular laryngitis. These cases have all been Hospital Out-patients, and have had no advantages except inoculation treatment. They do not, therefore, by any means express the results that might be obtained under more favourable circumstances, and they are here given to enable some opinion to be formed as to what results may be anticipated from inoculation in cases in which Sanatorium treatment is not available, and also to suggest consideration as to how far treatment by inoculation might be of benefit in addition to that given in Sanatoria.

In the 26 cases the results are as follows :—

Cured	4 cases
Much bettter.	9 ,,
Slightly better	3 ,,
Unchanged	10 ,,

By "unchanged" it is meant that the progress of the disease has not been arrested and the cases have continued to go down hill.

Of the four cases cured, one was affected in two apices, and two in one apex only; and of the cases much better, four are recorded as affected in one apex only, while of those in which the disease had been progressive, one had a cavity, one suffered from pneumothorax, one had two lobes affected and two only were affected in a single apex; so that as far as these records go, the smaller the lesion the better the prognosis, as might have been anticipated.

Age. The majority of cases are between 30 and 50. Up to 10 years of age there is one case, from 10 to 20, two cases, from 20 to 30, eight cases, from 30 to 40, seven cases; and over 40, five cases.

Dose. Doses have been very variable.

Minima.

1/120,000th	of a	milligramme	in	1 case
1/100,000th	,,	,,	,,	2 cases
between 1/100,000th and 1/50,000th	,,	,,	,,	8 ,,
between 1/50,000th and 1/20,000th	,,	,,	,,	7 ,,
between 1/12,000th and 1/8,000th	,,	,,	,,	3 ,,

Maxima.

Between 1/50,000th and 1/75,000th	of a	milligramme	in	4 cases
between 1/20,000th and 1/30,000th	,,	,,	,,	5 ,,
1/15,000th	,,	,,	,,	4 ,,
between 1/12,000th and 1/8,000th	,,	,,	,,	4 ,,
1/4,000th (and upwards)	,,	,,	,,	5 ,,

In the cases which have yielded good results the doses have been as follows:—

Minima.

Less than 1/50,000th	of a	milligramme	in	3 cases
1/50,000th	,,	,,	,,	4 ,,
1,50,000th to 1/20,000th	,,	,,	,,	5 ,,
above 1/20,000th	,,	,,	,,	1 case

Maxima.

Less than 1/20,000th	of a	milligramme	in	4 cases
from 1/20,000th to 1/10,000th	,,	,,	,,	4 ,,
above 1/10,000th	,,	,,	,,	5 ,,

Duration of the disease before treatment and duration of treatment. One case had lasted for weeks and one for months, ten cases are recorded as being of a year's duration. Fifteen cases were treated for less than a year and six for more.

Of the cases that have improved, one lost all physical signs and gained a stone in weight. Eight others also gained in weight. Subjective improvement is recorded in eight cases; in one there is a special note that a small dose produced the best results.

In the treatment of phthisis, therefore, the initial dose of tubercle vaccine should be 1/50,000th of a milligramme, which should be slowly increased to 1/15,000th of a milligramme. This dose may be not infrequently exceeded, but it is rarely necessary to exceed 1/4,000th of a milligramme. Individual susceptibility varies very much, and many persons require very small doses. Fairly good results may be anticipated in about half the cases treated. Treatment is of long duration and should be continued for at least a year.

In criticism of the treatment of these cases it is to be noted that very scant attention has been paid to infection with other organisms than the tubercle bacillus

Pleurisy and Empyema. One case of tubercular pleurisy has been treated, and three cases of empyema.

The case of pleurisy was much improved after a year's treatment. The dose varied from 1/8,000th to 1/5,000th of a milligramme.

Three cases of empyema with sinuses were treated from which organisms were obtained in great variety: pneumococcus, diphtheroid bacillus, influenza, staphylococcus and streptococcus. Of these two were cured, one of whom relapsed, and one was slightly better. It is obvious that such cases must be treated on their merits.

Whooping Cough. In 1906 Drs. Bordet and Gengou of Brussels discovered an organism which they regard as the cause of whooping cough. It is a small cocco-bacillus, non-motile, decolourised by Gram's method and of feeble staining properties. It is found in great quantity, and often in almost pure culture, in the bronchial mucus at the onset of the disease. It is present in 80 per cent. of cases of whooping cough.

Culture. This is a matter of great difficulty, particularly in the first cultures obtained on artificial media. It can be cultivated on serum agar and blood agar. It is difficult to separate from contaminating organisms. In appearance it is very like the influenza bacillus, although the two differ in cultural characteristics. The influenza bacillus will only grow in the presence of hæmoglobin; it comes to its full growth in twenty-four hours and only yields minute white colonies. Bordet's bacillus grows on serum agar; no growth is visible in twenty-four hours, but a growth far more profuse than that of influenza is found in forty-eight hours. The organisms can also be distinguished by means of serum reactions; these reactions are, however, matters of great difficulty to carry out, so difficult that they cannot be used for the control of inoculation treatment.

The organism produces endotoxins which can be obtained separate from the organisms by a method devised by Besredka.

This poison, when injected into animals, produced necrosis and extensive sloughing.

Bordet carried out some prophylactic inoculations with vaccines in the case of children exposed to infection with whooping cough. The result was unfortunate, as many of the children took the disease in a severe form. This was doubtless due to the fact that the dose given was too large, and in consequence a profound negative phase was produced with increased susceptibility.

Freeman (*Brit. Med. Journ.*, Oct. 9, 1909, and *Proc. Roy. Soc. Med.*, 1910) has worked out the curative treatment of whooping cough by means of vaccines. His methods are based upon clinical observations only, since after repeated trials he was unable to obtain serum reactions which he considered reliable owing to the technical difficulties involved. Considerable pains were, however, taken to avoid prejudice. He treated only alternate cases with the vaccine, every other case having been inoculated with salt solution without the knowledge of the patient or his parents, and the results were recorded without knowledge if a given case had been treated with vaccine or salt.

Results. He has divided his results into five classes: "much better," "better," "unchanged," "worse," "much worse." In an early series of experiments he found among the cases treated by vaccine:—

Much better	31	per cent.
Better	37·1	,,
Unchanged	15·7	,,
Worse	15·2	,,
Much worse	1	,,

Of the control cases inoculated with salt, he found:—

Much better	21·5	per cent.
Better	34·5	,,
Unchanged	23·9	,,
Worse	18·15	,,
Much worse	1·85	,,

In a later series of cases he found that the average duration of the disease among vaccinated children was 4·3 weeks, and in unvaccinated 7·4 weeks, a clear gain of three weeks to the vaccinated cases.

Dose. The doses of the first series varied from 2½ millions to 20 millions, having been worked up from minimal amounts. The dose now recommended is from 100 to 200 millions.

It should be borne in mind that only one organism has been experimented with in these observations. The influenza bacillus is also frequently present in these cases, and has doubtless considerable bearing on the condition in many instances, but with regard to Bordet's bacillus, Freeman's observations indicate that considerable benefit may be conferred by inoculation with the vaccine prepared from it. In individual cases a pneumococcus vaccine is often required.

Bronchial Asthma. Many persons who suffer from chronic bronchitis complain of attacks of spaśmodic dyspnœa as an especially distressing symptom. These attacks are generally called "Bronchial Asthma." They are precipitated by any exertion, and are also prone to occur at night and disturb sleep; the patients very frequently complain that they cannot lie flat, but have to be propped up in bed. Spasmodic dyspnœa occurs further in heart disease and uræmia, and also as a more or less independent affection sometimes called "true asthma." How far the latter is independent is a matter of dispute. The symptom seems to be the same thing in whatever disease it occurs, and it may be regarded as a reflex disturbance which is precipitated by a variety of causes.

The present writer (*Brit. Med. Journ.*, Oct. 9, 1909) has drawn attention to the fact that the condition of bronchia

asthma is associated in certain cases with a specific micro-organism, and is amenable to treatment by means of the corresponding vaccine.

In 1907 during some investigations into the bacteriology of chronic bronchitis, in the laboratory of the Inoculation Department at St. Mary's Hospital, a certain organism was isolated in nearly pure culture from the sputum of a female patient. Her opsonic index to this was taken, and since this was low, inoculation was suggested, to which the patient agreed. She was given a dose of 25 millions hypodermically, and was instructed to come back in two days. She suffered severely from bronchial asthma. On her return she said that though her cough was no better her breathing had been much relieved.

After this the same vaccine was used extensively among patients suffering from bronchial asthma in more than 100 cases. Of these a series of 52 were collected who gave the experiment a fair trial, that is, attended for an inoculation at least twice. The degrees of the disability at the outset and the improvement under treatment were very various, so that any very positive statement of the results would be arbitrary and misleading. But taking the results as a whole, 31 cases found some degree of improvement in the frequency, and 39 in the severity, of their attacks. Twenty-five have improved in their powers of taking exercise, and 29 have slept better. In some cases improvement was slight, and in others temporary. In four cases no improvement at all resulted. In all these cases the use of the general remedies for asthma was avoided as far as possible.

Asthma is a symptom which is liable to great spontaneous variation, and further, it is largely a subjective symptom, so that the records of results depend very much on patients' statements and have to be discounted as such. But when all due allowance is made, if in three instances out of four in a total of 50 cases, patients inoculated with this specific

vaccine find subjective improvement of the same symptom, that may be considered presumptive evidence that the symptom is due to infection with the corresponding organism.

The search for this organism in other cases has been disappointing. The sputum of about 100 cases has been examined, and the organism has only been found in one other instance, and from this specimen an efficient vaccine was made. Attempts at serum diagnosis were equally unsatisfactory. Although in the first case a low opsonic index was found, as a rule no valuable information was obtained by this means. The characters of the organism are the following:—It is a short bacillus with rounded ends about the size of a pair of *micrococci catarrhales*. The size, however, varies, short forms are found which appear like cocci, and occasionally long forms occur. It stains readily with basic aniline dyes and is decolourised by Gram's method, and there is marked polar staining, the unstained portion in the middle being so clear as to suggest that one is looking at a pair of diplococci. In an opsonic preparation, however, organisms which have undergone phagocytosis stain homogeneously and are seen to be bacillary in form. The organism has no visible capsule, is non-motile, and does not form spores. It grows readily on agar and the ordinary media, and its growth is not inhibited by the presence of bile salts. On agar the colonies are pale grey, circular and slightly raised. They vary in size, but as a rule are smaller than staphylococcus and larger than streptococcus colonies. In stroke culture they form a continuous line along the track of the needle with scattered colonies at its edge. The growth is soft, but less wet than that of Friedländer's bacillus. It is not tenacious and is readily emulsified. It grows best at 37° C., at which temperature there is a visible growth in six hours and a profuse one in twenty-four hours. A visible growth occurs in twenty-

four hours at 15° C. It does not liquefy gelatine, does not coagulate milk, and it does not form acid or gas in any of the ordinary sugars.

The organism is not clearly identified with any described in the text-books. In appearance and growth it is similar to Friedländer's bacillus, and may perhaps belong to the same group. Most organisms, however, allied to Friedländer's bacillus have some power of fermenting sugars. On the other hand, it may be allied to the *micrococcus catarrhalis*, under which name a large number of organisms are probably included which have not yet been definitely separated. Micrococci, apparently identical with the catarrhalis sometimes lose in successive sub-cultures the tenacious character present in early cultures and become easily emulsified. These considerations suggest that the organism perhaps belongs to the catarrhalis group.

A further series of observations were carried out in one of the London Infirmaries through the kindness of Mr. J. R. Lunn, F.R.C.S., on 15 cases, and as inmates of a Workhouse Infirmary for bronchitis and asthma, it will be understood that they were severe cases. Of these ten underwent some degree of improvement in the course of about a month's treatment.

If treatment is undertaken it can only be done in an empirical way. The initial dose should be 25 millions of the stock vaccine, which may be increased to 50 millions after a week. Fifty millions appears to be the most efficient dose, and may be given at weekly intervals for a period of six weeks. After this it may be continued at intervals of two weeks, and after another six weeks at monthly intervals. Treatment must be guided entirely by clinical indications. The only symptom likely to be benefited by inoculations with this vaccine is the asthma; the cough appears to be almost entirely unaffected.

Associated with asthma is the condition of hay-fever.

Hay-fever itself, in many instances at any rate, is due to intolerance of the nasal mucous membrane of some individuals to certain pollens. It is possible, however, that this condition of the mucous membrane may be due to an underlying bacterial infection. The writer has had one case from which an organism, giving the reactions of the *bacillus ærogenes*, was isolated in small numbers and inoculation with the corresponding vaccine was followed by marked improvement in the hay-fever; the patient was enabled to attend a military camp and sleep on a straw palliasse without discomfort. The only inference to be drawn from such a case is that hay fever may possibly be due to an underlying bacterial infection.

Chronic Bronchitis. Only a small number of records have been examined of cases of this disease treated by inoculation. The organisms involved are necessarily numerous and varied, and too few cases have yet been found to be due to any particular organism to allow of tabular analysis. Different cases have yielded pneumococcus in pure culture, or pneumococcus and catarrhalis, or pneumococcus and the bacillus septus (a diphtheroid) and the influenza bacillus. Others, again, yield Friedländer's bacillus or similar coliform organisms. Sometimes considerable improvement has resulted, in others no apparent benefit has been received. It is clear that all such cases should be considered on their merits, and that generalisation is impossible when so much variety exists among the infecting organisms.

Nasal Discharge. Five cases of chronic nasal discharge have been treated; here, again, great variety of infecting organisms has been found, of which the chief are Friedländer's bacillus, pneumococcus, micrococcus catarrhalis, staphylococcus and streptococcus. Two cases are much improved, one has lost a fetor of long duration, two are

slightly improved, and one is unchanged. Here, again, if treatment by inoculation is contemplated, all cases must be treated on their individual merits.

Pneumonia. Twenty-four cases of lobar and primary lobular pneumonia have been treated by Drs. Willcox and Parry Morgan (*B.M.J.*, Oct. 9th, 1909). In most cases pneumococcus was found, but in two cases streptococcus occurred in pure culture and once the influenza bacillus. In a later communication (*Proc. Roy. Soc. Med.* Vol. III., Oct., 1910) Dr. Parry Morgan has reported a total number of 43 cases treated with two deaths, one from nephritis.

The authors are careful to insist that vaccine therapy is not to replace the approved methods of treatment, but is to be given in addition to it. They recommend the following routine :—

It is important that vaccine therapy should be begun at the earliest possible moment, an inoculation of 20 to 30 millions of stock pneumococcus vaccine should therefore be given on diagnosis. Search is then made for the infecting organism. It may be obtained from the sputum by planting out on serum-agar, but in this case sub-cultures are always required to obtain a pure growth, and two or three days' delay is therefore unavoidable. Organisms may also be obtained by blood culture, but blood cultures are not infrequently sterile.

A further method, and one which the authors have found especially useful, is by puncture of the lung; a few drops of sterile broth culture are taken into the syringe, the needle is passed into the consolidated lung, some, not all, of the broth is injected and immediately withdrawn. By this means a pure culture is frequently obtained and sub-cultures are not required, a vaccine being made from the primary growth. When an autogenous vaccine is prepared

a second inoculation should be given if possible in twenty-four or forty-eight hours, and may be repeated at intervals of two or three days if required. The dose should be reduced as the *crisis* is approached, since the susceptibility of the patient appears to be increased at that period. Doses for children should be reduced in proportion to their age. The writers are guided entirely by the clinical indications, since the technical difficulties in the estimation of the opsonic index to the pneumococcus are considerable.

The authors write of the clinical results of inoculation as follows: "The duration of the disease appeared in some cases to have been shortened; thus, out of our 24 cases, in one the *crisis* occurred on the second day, in one on the third day, in one on the fourth day, and in three on the fifth day after the onset."

"In some cases instead of a fall in temperature by *crisis*, the fever subsided by *lysis*; this happened in eight of our cases, a rather larger proportion, we think, than would have been the case without vaccine treatment."

CHAPTER XII

DISEASES OF JOINTS AND BONE: DISEASE OF LYMPHATIC GLANDS: DISEASES OF THE GENITO-URINARY TRACT: DISEASES OF THE EYE AND EAR

Arthritis. The largest number of cases of infective arthritis treated by inoculation whose records have been examined, have been due to either tubercle or gonococcus, a few others have been due to streptococci and staphylococci.

Tubercular Arthritis. This condition has been investigated by Maynard Smith (*British Medical Journal*, Oct. 9, 1909). The records of 70 cases were examined and the patients were requested to present themselves for examination, and 34 were examined. As is always the case in such investigations, the patients who attended were those who had received benefit from treatment, and it is from these 34 cases that the following account is made out:

Age. Up to 10 years of age, 13 cases; between 10 and 20, ten cases; between 20 and 30, six cases, and over 30, five cases. It is therefore chiefly a disease of young persons.

Duration. The cases were all chronic: none of them were of less than a year's duration before treatment

began; 16 of them were of less than three years' and 18 of more than three years' standing. Nearly all underwent inoculation treatment on the recommendation of a surgeon, so that in addition to inoculation, they were receiving appropriate treatment by splints, massage and other surgical methods.

Site of the lesions. Six were in the upper extremity—shoulder, elbow or wrist—28 in the lower extremity, and of these 16 were in the knee joint.

Duration of treatment. This also was long continued. Fifteen cases were under treatment for a period of months, and 19 for periods exceeding one year.

Dose. The doses vary from 1/12,000th of a milligramme minimum, to 1/5,000th maximum. A large number of the cases had sinuses, and many of these had secondary infection with the organisms which commonly occur on ulcerated surfaces. These were treated with appropriate vaccines.

Results. The results were as follows:—

Cured	10 cases
Much better	18 ,,
Better	4 ,,
Unchanged	2 ,,

Relapse was recorded in two instances. It should be noted again that these are to be considered to some extent as "picked" cases. Some of the results obtained seem to have been extraordinarily good, in several cases in which one joint had been affected with definite physical signs, such as swelling, wasting or immobility at the period when treatment was begun, at the time of examination no difference could be detected on the two sides; this is recorded in nine instances.

In the treatment of tubercular arthritis all approved surgical methods should be carefully carried out, and in addition to that inoculation should be undertaken beginning with doses

of 1/12,000th of a milligramme at intervals of ten days. These should be gradually increased; it is rarely necessary to exceed 1/5,000th of a milligramme. Treatment is of long duration, and in the majority of cases must be carried out for at least a year.

Gonococcal arthritis. Records of 23 cases have been examined.

Age. This disorder, as a rule, occurs much later in life than tubercular arthritis. No cases in this series were under 20 years of age; twelve occurred between 20 and 30; seven between 30 and 40, and three over 40.

Duration. The cases were chiefly chronic, though in several treatment was begun at an earlier stage than in the tubercular cases. Four cases came under treatment after a period of weeks, five after a period of months, and nine after a period of years.

Site. Joints of the upper extremity were affected in four instances; of the lower extremity in thirteen instances, and many joints were involved in six instances. In four cases mixed infections were treated.

Dose.	
Minima.	
1 million	in 6 cases
2 to 2½ millions	in 7 ,,
3 ,,	,, 1 case
5 ,,	,, 6 cases
10 ,,	,, 1 case
Maxima.	
4 millions	in 1 case
5 ,,	,, 1 case
10 ,,	,, 2 cases
15 ,,	,, 3 ,,
20 ,,	,, 5 ,,
25 ,,	,, 2 ,,
30 ,,	,, 1 case
35 ,,	,, 1 ,,
40 ,,	,, 1 ,,
45 ,,	,, 1 ,,
50 ,,	,, 2 cases

Results are as follows :—

Cured	3 cases
Much better	7 ,,
Better	13 ,,

None recorded as unchanged or worse.

Of the ten cases which are cured or much better, one had been under treatment for weeks ; seven of them were under treatment for months and two for years.

The *minimal dose* among these cases was 1 million four times, 2 to $2\frac{1}{2}$ millions four times, 5 millions twice ; and the *maximal dose*, 4 millions once, 5 millions once, 10 millions once, 15 millions twice, 20 millions once, 35 millions once, 40 millions once and 50 millions once.

In the treatment of gonococcal arthritis, therefore, good results may be anticipated in half the cases. With regard to dose, individuals vary very much in their susceptibility, and beyond the fact that doses must be kept very small it is difficult to lay down precise rules. Probably $2\frac{1}{2}$ millions is a safe initial dose in any but extremely acute cases. In these, one million should not be exceeded at first. Treatment will have to be continued for about a year, and the dose should be gradually increased. The maximum dose also depends on individual susceptibility : 50 millions is hardly ever exceeded.

Other cases of arthritis. A few records are available of cases of arthritis successfully treated by streptococcus vaccine. Of these, two are cases associated with oral sepsis. From the gums a streptococcus was isolated and the patients were inoculated with doses beginning with 20 and going up to 50 millions. In one instance marked improvement in pain and mobility was arrived at, and in one slight improvement. Other cases have been treated in which with a similar condition of the mouth and the isolation of similar organisms therefrom, no improvement has been received, the vaccine appearing to produce no effect whatever upon the condition.

In a few other instances of chronic arthritis, organisms have been obtained from the urine, more particularly a coccus which partakes of the characters of both the streptococcus and staphylococcus, that is to say, in the film from an apparently pure culture some bacteria were found in groups like staphylococcus, while others apparently identical in their individual components were found in chains. In two instances patients have been apparently considerably benefited by vaccines made of those organisms obtained from their urine. Treatment has in each case been continued for about six weeks, and at the time of treatment no great change was found, but during the subsequent months the arthritis has been considerably relieved. Both were in cases of women of middle age.

Bone. Twenty-four cases of disease of bone, without involvement of joints, have been treated. With one exception these have all been tubercular infections, and additional organisms were present in ten cases. Staphylococcus has been found nine times, once in association with a streptococcus, and in one case there was a mixed infection with the bacillus coli and a diphtheroid bacillus.

Age. Up to the age of ten there have been 11 cases, between 10 and 20, five cases; between 20 and 30, one case, and above 30, five cases.

Duration. Two cases had lasted for a period of weeks before treatment began; six for a period of months; five for a period of years.

Twelve were under treatment for a period of months and ten for a period exceeding a year.

Site. The upper extremity has been affected four times, the lower extremity ten times, the spine eight times, and the mastoid twice.

Results.

Cured	2 cases
Much better	7 ,,
Better	8 ,,
Unchanged	1 case

Of the nine cases cured and much better, six were in children under ten years of age. They were treated with the following doses:

Minima.

1/100,000th	of a	milligramme	in	1 case
1/30,000th	,,	,,	,,	1 ,,
1/20,000th	,,	,,	,,	3 cases
1/15,000th	,,	,,	,,	2 ,,
1/10,000th	,,	,,	,,	1 case
1/8,000th	,,	,,	,,	1 ,,

Maxima.

1/15,000th	of a	milligramme	in	3 cases
1/8,000th	,,	,,	,,	3 ,,
1/6,000th	,,	,,	,,	2 ,,
1/4,000th	,,	,,	,,	1 case

Six were under treatment for a period of months, three for a period of years. Cases of secondary infection were treated by appropriate vaccines, as in cases of ulcer.

In tubercular disease of bone good results may be anticipated in from one-third to one-half of cases treated, and the younger the patient the better the probable result. The doses are fairly large except in very young children. The initial dose should be 1/20,000th of a milligramme, and may be increased to 1/6,000th. Treatment will probably last from six months to a year.

Lymphatic Glands. The only disease of lymphatic glands against which inoculation treatment has been seriously directed is tuberculosis. The records of 155 cases of tubercular lymphatic glands have been studied, and 87 of the patients have been clinically examined. The following account (*British Medical Journal*, Aug. 28, 1909) is based upon the examination of these 87 cases. In eight of them inoculation was employed as an adjunct to removal by

operation, in every case with satisfactory results and with no recorded recurrence.

There remain 79 cases in which inoculation has been the chief method of treatment. The cases were, on the whole, decidedly bad ones. Of the 79 cases, 43, or more than half, had been operated on previously; and 21, or one-quarter, had had more than one operation; five of them had had ten operations or more.

Site. In ten of the cases the affected glands were either in the axilla, arm, or groin; in all the rest they were in the neck.

Dose. The minimum dose is usually 1/15,000th or 1/20,000th of a milligramme, the smaller dose being always used for children. The maximum dose for children under five is 1/10,000th; for older children and adults it rarely exceeds 1/4,000th; in a few instances 1/3,000th has been used, and 1/2,000th is recorded. Secondary infections were treated with appropriate vaccines. The importance of local treatment in certain circumstances in this condition is referred to below.

Age. The age incidence is as follows:

Up to the age of	5	years	there	were	8	cases	
between	5	and	10	years	16	,,	
,,	10	,,	15	,,	10	,,	
,,	15	,,	20	,,	13	,,	
,,	20	,,	25	,,	4	,,	
,,	25	,,	30	,,	7	,,	

Results. These are classified in the following way: When glands have disappeared altogether, or have been reduced to a size not larger than cherry-stones, and then remain hard and do not vary in size, they are considered "cured." When there is marked diminution, but the glands are of larger size than the foregoing—say as large as raisins—they are considered "much better." When the reduction is slight only, they are called "better," and others are described as "unchanged" or "worse." Relapse after attain-

ing to some degree of improvement has occurred in 11 cases. In these 79 cases results are as follows :—

Cured	27
Much better	22
Better	18
Unchanged	8
Worse	4

The reasons for these variations in result have been sought for, and the age of the patients, the duration of the disease before treatment, the period of treatment, the presence of tubercle elsewhere, the presence of secondary infections, and the dose, have all been considered. The same variations of dose have been employed in every class; the different results cannot depend on this. Tuberculous lesions elsewhere as well as in the glands are rare in this series (only 12 in all), and these are evenly distributed; they do not much affect the prognosis. Secondary infections also occur irregularly in all grades of success. The duration of the disease before treatment was begun and the duration of treatment have both a slight relation to the results, the advantage being on the side of short duration and long treatment; but the difference is not sufficiently marked for either to be a potent cause.

Relation of age to results. The percentage of cases yielding good results, namely, those cured and much better, found in each five-year period up to 30 years of age is as follows :—

Up to 5	years	of age	75	per	cent.	cured	or	much	better
From 5	to 10	years	50	,,	,,	,,	,,		
,, 10	,, 15	,,	50	,,	,,	,,	,,		
,, 15	,, 20	,,	84	,,	,,	,,	,,		
,, 20	,, 25	,,	70	,,	,,	,,	,,		
,, 25	,, 30	,,	43	,,	,,	,,	,,		

The figures indicate clearly the important influence which age has upon results in the disease. Immunisation occurs satisfactorily in the first period; there is then a fall till after the age of puberty, after which there is a marked rise in

resistance, which is well maintained for ten years. During the period from 10 to 15, not only is the number of improved cases small, but the number of failures is greater than at any other time.

Reference should be made to that series of cases in which the glands have entirely disappeared under treatment. These are eleven in number; with four exceptions they were all between the ages of 18 and 23, seven of them had had enlarged glands for less than a year, five for less than six months, and only two for more than a year. It would seem, then, that the most likely cases for complete recovery are those occurring at about the age of 20, and coming under treatment early; such recovery occurs in a few months; in only three instances has treatment exceeded a year's duration in these cases.

In any average eight cases, therefore, in which inoculation is adopted, with correct dosage, five will probably show marked improvement, of whom two or three will be cured; two will probably improve slightly, and one or two will fail. The best results are to be anticipated in young children and in young adults from 15 to 25, after which they gradually deteriorate; the worst results occur between the ages of 10 and 15. Treatment must be expected to last a year. Success will not be attained unless secondary infections are appropriately treated.

In the treatment of tubercular glands, besides the inoculation of vaccines, local treatment requires special attention. It is of the first importance that sources of irritation, such as carious teeth, pediculi, or aural discharge shall be appropriately dealt with. Further, as was pointed out in an earlier chapter, the presence of stagnant fluids at the site of infection is a hindrance to immunisation, and such fluid when present should be removed. In glands which are near enough to the surface for the presence of fluid to be detected by palpation, the fluid should be drawn

off by a syringe. A large syringe of the capacity of 5, 10, or 20 c.c. should be used with a large, but very sharp needle. The skin is washed with absolute alcohol and the needle thrust into the gland, and the pus aspirated off. If there is more fluid present than can be withdrawn in one syringeful, the syringe may be disengaged from the needle, emptied, and re-applied without withdrawing the latter. After withdrawal the puncture should be dressed with a small dry dressing; if any liquid is applied it should be lead lotion, in order to harden the skin as much as possible. In this way the establishment of a sinus can often be avoided. Aspiration may be practised many times on the same gland, and is attended with good results: it is recorded in nine cases in this series, four of them among those completely cured.

The Genito-urinary Tract. Thirty-four cases of tubercular disease of the genito-urinary tract have been treated, nine of the testis, eight of the kidney, and 17 of the bladder.

Age. The age of incidence is higher than in the case of glands or ulcer, nearly all cases occurring between 20 and 40, and being evenly distributed through that period.

Results. Infection of the Testis. Three cases are cured, two much better, two better, and the rest doubtful.

Infection of the Kidneys. Two cases are cured, two much better, three better, and one dead. The death occurred after childbirth, and at a time when the renal symptoms were improving.

Infection of the Bladder. Two cases are cured, four much better, eight better,—that is, have had some relief of either their pains or their frequency of micturition—one is no better, one worse, and one unknown.

Relapse has occurred in five cases.

Secondary infections, chiefly by the bacillus coli, have been treated in 13 cases.

The dose should be a very small one at the outset, in 10 out of 15 of the successful cases the initial dose has been less than 1/15,000 mg., often 1/25,000; it may in time be brought as high as 1/4,000, but very bad results may arise through excessive initial doses. Treatment in the successful cases has averaged 1 year and 2 months, though five cases have been treated for 6 months or less.

In tubercular disease of the genito-urinary tract great improvement may be anticipated in three cases of seven, and slight improvement in two more. The initial dose should not exceed 1/25,000 mg., and treatment will probably last a year. Secondary infections occur in about half the cases.

Non-tubercular infections of the urinary tract. Cases of cystitis are frequently amenable to treatment by vaccine therapy. Many different organisms are found, of which the commonest are those of the coli group. The *bacillus coli communis* and the *bacillus acidi lactici* are fairly frequently found. A specific vaccine must always be made. The results are variable: cases of long standing sometimes respond remarkably soon, and after one or two inoculations of small doses will immediately clear up. Others are extremely resistant, and when this is the case, it is always well to consider whether there is any condition, such as the presence of a stone, which is keeping up the infection. There are cases which on clinical grounds are diagnosed as tubercular, but in the urine of which tubercle bacilli are never found but only coliform organisms. When such cases are inoculated with the coliform vaccine, little, if any, improvement occurs, and the bacteria persist in the urine, the reason being probably that they are tubercular and that some part of the urinary system contains masses of dead matter which act as foreign bodies and prevent healing.

Cases are occasionally met with of bacilluria without actual

cystitis, that is to say, organisms are obtained from culture of catheter specimens of the urine but no pus is present. The condition sometimes follows abdominal operations. In these cases great improvement and cure often follow the exhibition of a vaccine.

In all cases of cystitis the bacteriology of the urine requires very careful investigation. Such cases as the following are frequently met with :—

A child of eight suffered from symptoms pointing to either stone or tuberculosis. The bladder was sounded for stone with negative results. Tubercle bacilli were present in the urine. Inoculation with tubercle was undertaken, and in the course of a few weeks the tubercle bacilli disappeared with some improvement in the clinical condition, but without cure of the cystitis. Profuse growth of the bacillus coli was obtained from the urine, and the patient's opsonic index to this was found to be slightly above normal. He was inoculated with $2\frac{1}{2}$ millions, whereupon the index promptly fell to ·2, but subsequently recovered, and after a few inoculations the bacillus coli entirely disappeared from the urine. The cystitis, however, persisted, and a further bacteriological examination of the urine was made, with the result that a tetrad organism was obtained from it, and inoculations with this were begun, and ended in complete recovery. About a year after this a slight relapse occurred and a tetrad organism was again obtained from the urine. After a few inoculations the condition again cleared up.

Diseases of the Eye. A few records of the following diseases have been examined: non-tubercular conjunctivitis, keratitis and corneal ulcer, tubercular keratitis, choroiditis, retinitis and iritis.

Non-tubercular conjunctivitis. Four cases; in three of these staphylococcus was present, and in one streptococcus.

Of the staphylococcal cases, one is described as "cured," one as "nearly well" with regard to the conjunctivitis, but with the nebula unchanged, and the third is described as "improved."

Two of them were under treatment for periods of three weeks, and one for a period of three months, irregularly.

The doses were small, varying from 50 to 200 millions.

The streptococcal case was a chronic one of ten years duration and was under treatment for ten weeks. It was considered to be improved by the oculist in charge, but varied a good deal.

Tubercular phlyctenular keratitis. Nine cases: The cases were chiefly chronic, though the records on this head are imperfect. Six of them were tubercular only, three had additional infections. Five of them were much improved, three improved, and one, in which a large variety of contaminating organisms were present, was unchanged.

Dose. The initial dose was usually 1/20,000th except in the case of small children, and the highest dose recorded is 1/6,000th of a milligramme. The majority of cases were under treatment from three to six months. Staphylococcal infections, when present, were treated with doses varying from 50 to 200 millions.

Two cases of tubercle of the choroid and one of tubercle of the retina have been treated with similar doses. One is described as much better and two as slightly improved.

Iris. One case of tubercle of the iris has been treated for seven months with doses of from 1/20,000th to 1/10,000th of a milligramme, with improvement and to the satisfaction of the ophthalmic surgeon in charge. One case of staphylococcal infection of the iris associated with osteo-myelitis was very much improved and nearly cured in three months, doses of from 50 millions to 100 millions being employed.

Two other cases of choroiditis associated with pyorrhœa

alveolaris have been treated, one of which improved considerably with a vaccine of the streptococcus of the mouth, receiving doses of 30 millions. The other case improved slightly, but this was probably not referable to the vaccine.

Aural discharge. A few cases of otorrhœa have been treated. A very large variety of organisms were found, frequently of the coli group, including the bacillus pyocyaneus; diphtheroid organisms have also often been found. Some cases improved considerably, but all such cases must be treated on their merits.

APPENDIX

TECHNIQUE

For the purposes of vaccine therapy and bacteriological diagnosis, the ordinary appliances of a Bacteriological Laboratory are required, and also some special apparatus. Some description is here given of the latter, and of the technique employed in its use, under the following heads :—

1. Large apparatus.
2. Minor articles which the vaccine-therapist will make or adapt for himself, with the methods of their manufacture.
3. Technique.

Section I.

The large apparatus required consists of an opsonic incubator, a centrifuge, a shaker, a vaccine steriliser and a blow-pipe.

Incubator. Besides the ordinary bacteriological incubator, a second incubator, maintained at a temperature of 37° C., of a special pattern for use in the estimation of the opsonic index is a very great accommodation though not absolutely essential. The usual pattern is that devised by Freeman, and consists of a rectangular metal box about 10 × 7 inches square and 3 inches deep, containing water. It is maintained at the required temperature by gas-burners placed beneath it, the gas supply being controlled by a thermo-regulator immersed in the water. The box is pierced from

front to back by a row of hollow horizontal cylinders into which a series of pipettes can be passed. A temperature of 37° C. is maintained in the cylinders, and the pipettes can be introduced and withdrawn at any required moment without the loss of time involved in opening and shutting the door of an ordinary biological incubator. Since it is frequently desirable to estimate a large number of indices at one sitting and the pipettes are filled and passed into the incubator at intervals of one minute, and each requires to be withdrawn after the same period of incubation, this apparatus is a great convenience.

A *Centrifuge* is also required which will take sedimentation tubes 5 centimetres long and 1 centimetre in diameter. It may be driven either by hand or machinery; for the latter purpose Martin's pattern is exceedingly good, it consists of a circular horizontal plate, under which the tubes are hung. Such instruments may be driven by water or electricity; high speeds are required.

Shaker. Vaccines also require to be agitated in order to separate the organisms into an even suspension. This may be done by hand or if desired by a mechanical contrivance. If the centrifuge is driven by a motor, a simple shaker can be driven by the same power. Such an instrument consists of a vertical wheel, with an eccentric rod moving a second rod, which is hinged to the bench at its lower end, and has a vice for holding test-tubes at its upper end.

Vaccine Steriliser. This is required for heating the vaccines. Various expensive apparatus are obtainable, but for practical purposes all that is required is a metal vessel containing water, in which the sealed test-tubes containing vaccine can be immersed. The vessel must stand either on feet or on a tripod to allow of its being heated by gas-burners. It is best to regulate the heat by a thermo-regulator set to keep a temperature of 60° C., but if a large bulk of water is used (two gallons) this can be kept at the required temperature for an hour with very little trouble.

Blow-pipe. This is an instrument with which a stream of air is driven by a bellows through a stream of lighted coal-gas, so that great heat can be concentrated on a small area.

Section II.

The use of the Blow-pipe. The following articles are conveniently made with this instrument from soft glass tubing:—(1) Blood capsules. (2) Pipettes. (3) Sedimentation tubes.

The blow-pipe is also required for sealing test-tubes containing vaccines.

The glass tubing requires to be cut into suitable lengths, for which purpose a glass-cutting knife is required. This may be made from a good pocket knife-blade by hardening the steel. The blade is heated in the blow-pipe flame to an orange heat and then immersed in cold water, after which it is sharpened upon a sand-stone. It is thus really converted into a fine file of hardened steel, with which the surface of the glass tubing may be rasped; if snapped across it will then break at the rasped point.

Three different bores of glass tubing are required having an outside diameter of 4, 6, and 8 millimetres respectively. The thickness of the tubing, which, however, cannot be obtained quite uniform, should be about 0·5 millimetre.

For glass-blowing purposes turn the gas- and air-cocks of the blow-pipe wide open, and work the bellows with the foot until an even stream of air is driven through the flame. Then, leaving the air cock on full and maintaining the same air-pressure, slowly turn down the gas-cock. The flame is now reduced in width but is blown with greater force—becomes so to speak a "stiffer" flame. Flames of different widths are required for different purposes, but as a rule a flame which appears to be about 15 centimetres long and 1 to 2 centimetres wide is the most useful.

There are two rules to be observed in glass-blowing: First, *when in the flame, the glass is to be heated only and not moulded.* The tubing must, however, be very thoroughly heated and should be quite red before it is withdrawn, and it must be rotated so that the whole circumference is equally exposed to the flame at the point heated. But it must be withdrawn from the flame before any moulding is done. Secondly, *the glass must be moulded by quite steady movements of the hands.* There is ample time for all the manipulation required after the glass is withdrawn from the flame: no time must be wasted, but there is no need for snatching or jerky movements.

Wright's blood-capsule. This is made from glass tubing of 4 millimetres diameter. When finished it consists of a glass tube about 6 centimetres (2½ inches) long, wide in the middle, but with both its ends drawn out fine, one end being straight, and the other bent into a curve (see Plate I, fig. *f*). It is made thus:—A piece of tubing about a foot long is selected. One end is held in the right hand, the left hand holding the tubing at a distance of 3 or 4 inches from the right. The tubing is heated by a narrow flame between the two hands, care being taken to revolve the tube in the flame so that it is evenly heated. When malleable it is withdrawn from the flame, and the heated part stretched by drawing the hands apart in a straight line. The tube is thus lengthened, and the movement should be continued until the heated portion is about 6 inches long (Plate I, fig. *b*). The movement is made with the even steadiness with which one would stretch a piece of elastic. The tube is now broken or fused in the middle of the thin part (fig. *c*). Reject the end held in the right hand: the end held in the left hand is now a long glass tube of which one end is drawn fine. Hold the fine end in the right hand and the unheated tubing in the left hand, the hands being 3 or 4 inches apart, palms downwards. Heat the tube at a point about 1 inch to the left of the point where it begins to taper, rotating it as before. When hot withdraw from the flame and draw out as before, then move the right wrist in such a way as to bend the right-hand extremity of the thin part into a curve (fig. *d*). This is done chiefly by a movement of supination of the right wrist, intensified by bringing the right elbow towards the body. When the tube has been withdrawn and pulled out, the hottest part is at the point at which the least tapering has occurred, so that when the right wrist is moved in this manner, it will be found that the tube will only bend at the right-hand extremity of the part which has been recently heated. If the left hand is kept steady, no bending need occur at the left-hand end of the heated portion. The middle of the thin part may now be broken, and it will be found that the capsule of the pattern described has been made (fig. *e*). The curve of the capsule should be a wide one, as shown in the drawing, and the diameter of the capillary part should be at least one millimetre. Beginners usually make a curve

and bore which are too small because they use too narrow a flame. The longer the heated portion of the tube, the wider will be the curve produced on bending, so that a fairly wide flame should be employed when heating for this purpose.

Other capsules may be made by heating the tube an inch further down and repeating the process as before. The two ends of the capsules so made will be inconveniently long, and it is usual to cut the straight end so that the tapering part is about $1\frac{1}{2}$ inches long, and the curved part so that it is about half an inch long. The curved end is rasped across with the glass-cutting knife and snapped, which thus leaves a square-cut end. The straight end is conveniently made into a needle for puncturing the finger and drawing blood. The thin part is held in both hands in the by-pass flame of a Bunsen burner and the heated part rapidly pulled out. The hair-fine tube thus made is broken off and a sharp point is left at the end of the capsule (fig. *f*).

Wright's pipette. This is used in the estimation of the opsonic index, and it is made from tubing of 6 millimetres diameter. When finished it is a cylinder about $1\frac{1}{2}$ inches in length, of which one end is drawn into a capillary tube (Plate I, fig. *k*). The capillary end is rasped and snapped off square. It is made as follows:—A piece of 6 centimetre glass tubing is cut into 3 inch lengths (fig. *g*). A length is held between the two hands and the middle part of it is heated by rotation in the blow-pipe flame. When hot it is withdrawn from the flame and pulled out slowly and steadily till the hands are about 1 foot apart (fig. *h*). The middle of the capillary tube is then fused and two pipettes are thus made (fig. *j*). As before, the hottest portion of the tube is that at the point where it *begins* to taper, so that if the rate of stretching is increased—that is, if the hands begin to separate slowly and are then separated quickly because the pipette is not going to be long enough, stretching will occur at a point near the wide end, which will then become the thinnest part of the tube. Such a tube lacks rigidity and is springy, and it is difficult to make movements of precision with its point, therefore in making a pipette it is important that the hands shall be separated at an even rate. Before use the pipette is cut off with the

glass-cutting knife so that the capillary portion is 10 centimetres (4 inches) long (fig. *k*). The diameter of the capillary end of the tube should be between a half and one millimetre.

For various purposes a "bulbous pipette" is required, that is, a pipette containing a dilated chamber above the capillary stem (Plate I., fig. *l*). It is made as follows:—A thick piece of tubing six inches long is selected, and a pipette is made in the ordinary way except that the unheated portion of the tube is about 4 inches long. The capillary end is sealed in the flame. The tube is then heated at a point just above that where tapering begins till it is very hot and malleable. It is withdrawn from the flame, the wide end is placed in the mouth, and vigorous and steady blowing is maintained down the tube. The heated portion will thus be blown into a bulb. Care must be taken not to blow this too large or the glass will become too thin and brittle.

Sedimentation-tubes. These are intended for use in the centrifuge. They are made from tubing of 8 centimetres diameter and it is important that they should be made in pairs of equal length in order to insure that the centrifuge shall be properly balanced. The tubing is cut into 4 inch lengths and heated and drawn out, and fused off as if for making pipettes (Plate II, figs. *a*, *b*, *c*). Each pipette is then fused off at the point where it begins to taper in order to make a miniature test-tube, the thin end being rejected. The end of the wide tube is thus sealed up (fig. *d*) into a rough conical extremity. The extreme end of this is again heated, and when hot the open end is placed in the mouth and gentle blowing is maintained; with care this end may be blown out to resemble that of a test-tube; it is not desirable to render the end at all bulbous (fig. *e*).

Sealing of Test-tubes. In the course of preparation of vaccines, it is necessary to seal the suspension of bacteria in test-tubes. This is done as follows:—The test-tube must be not more than half full (Plate II, fig. *f*). It is held in the left hand in a nearly vertical position, and a broad yellow flame from the blow-pipe is allowed to play upon the glass an inch or two above the liquid. This is of relatively moderate heat and is intended to dry the tube before high temperature is applied. The lower end of the

tube is held in the left hand, and the open end in the right. The gas is now turned down, so that a flame apparently about a centimetre in width is obtained. This is driven forcibly against the dry part of the test-tube. The tube must be rapidly revolved in order to heat the glass as evenly as possible. It may be found that the fingers of the right hand become scorched in this process. If that is so, a piece of ordinary tubing should be fused on to the mouth of the test-tube to afford greater length. When the glass at the point heated has turned red in its whole circumference, the test-tube is withdrawn from the flame, and care being taken to allow no fluid to reach the heated part, the right hand is drawn directly away from the left, and the heated portion of the tube is thus pulled out till the whole tube is about 18 inches long (fig. *g*). The thin portion is fused in the flame, leaving a closed tube about 9 inches long (fig. *k*), which must be kept in a vertical position until quite cool. It is a good plan to cut off the tip of this tube with the glass-cutting knife and seal the open end in a Bunsen flame, when a neatly-rounded extremity can be made (figs. *h*, *j*).

Section III.

A. *Technique of the Opsonic Index.* For this the opsonic incubator described above is desirable, and the centrifuge and the following small materials are required:—Sterilised solutions of sodium citrate 1·5 per cent., and sodium chloride 1 per cent.; two sedimentation tubes, blood capsules, pipettes, an indiarubber teat accurately fitting the wide ends of the pipettes, microscope slides of good quality 3 ins. × 1 in., a Bunsen burner with a by-pass, very fine emery paper (00 Hubert), a paraffin pencil, a microscope with a 1/12th oil-immersion lens and a No. 4 eye-piece. The biological requisites are: healthy blood corpuscles washed free of serum, an emulsion of bacteria in pure culture, and the serum of healthy, and also of infected, persons.

The object of the experiment is to compare the average number of bacteria which can be ingested by healthy leucocytes when incubated with different sera, healthy and pathological. Samples of the same leucocytes and of the

same bacteria are used in each experiment, and incubated for the same period; the varying factor in each case is the serum.

Serum is obtained from blood drawn from the finger. If cold, the finger of the person supplying the specimen may be first warmed before the fire or by friction, or may be congested by holding the hand low or swinging it round. A finger-bandage is now wrapped tightly round the index finger from the root to the tip, the last turn just covering the distal joint. The finger is now flexed and will be seen to be congested. A Wright's capsule is then taken (*vide supra*), and with the sharp end three or four punctures are made in the skin at the root of the nail. These need not be deep, but should be deep enough for a drop of blood to appear immediately. If gentle pressure is made on the nail a large drop of blood will gradually be collected. *The sharp point of the capsule is now broken off* and the curved end is introduced into the drop. Blood will flow by capillarity into the capsule. If the other end of the capsule is kept pointing downwards, the flow of blood will be assisted by syphon action. It is important to collect enough blood, the body of the capsule must be at least half full; the actual amount of blood required is about 1/10 c.c. When the capsule is full enough the open end is sealed in the by-pass flame, and when withdrawn from the flame, the blood will be observed to retract from the curved end, as the air cools between the blood and the closed end. If it is necessary to send such a specimen by post it is best to shake the blood down to the closed end, when the open end may be either fused or closed with sealing wax. In sealing a capsule great care must be taken not to heat the blood, or the value of the serum will be completely destroyed. When the blood has been drawn the capsule may be placed in the incubator, since coagulation proceeds more rapidly at 37° C. than at ordinary room temperature. The blood will coagulate in about five minutes, and in about twenty minutes the clot will have contracted sufficiently to allow of the serum being easily withdrawn, without taking up any corpuscles. When moved the capsule should always be put down with the same side uppermost as before, since the clot sinks to the bottom, and if overturned there will be difficulty in withdrawing

clear serum. If necessary the separation of the clot may be hastened by centrifugalisation.

Preparation of blood-corpuscles. A sedimentation tube is taken, and filled with solution of citrate of soda to within half an inch of the top. The finger or thumb of a healthy person is congested as before described, and several punctures are made immediately below the nail. When the blood flows it is collected in the sedimentation tube until the latter is full. The tube is now closed with the finger and inverted several times to mix the blood with the solution. By this means coagulation is prevented. The second sedimentation tube is filled with water, and the two are placed in opposite tubes of the centrifuge, which is made to revolve at a high speed. In a few minutes, varying with the speed of the centrifuge, the blood corpuscles will be driven to the bottom of the tube, the serum being mixed with the supernatant fluid. The tubes should not be in the centrifuge longer than necessary, as the corpuscles are not improved by being jammed at the bottom of the tube by prolonged revolution. The supernatant fluid is drawn off by means of a bulbous pipette, the corpuscles being left behind. The tube is now filled up with 1 per cent. salt solution and shaken. The corpuscles are thus washed in salt-solution, they are then "centrifuged" again, and the supernatant fluid is again drawn off, fresh salt-solution is added and the process repeated once more, and the supernatant salt-solution is again withdrawn and rejected. The blood corpuscles have now been three times washed and they are regarded as being quite free of serum. Red and white corpuscles are found to be intimately mixed together throughout the mass. The leucocytes are not confined to the upper part.

Emulsion. For the preparation of an emulsion of any bacteria except tubercle bacilli the following appliances are required:—(1) A recent culture of the organism under investigation on a solid medium, if possible a thick culture on agar of twenty-four hours' growth. (2) One per cent. salt-solution. (3) Flat-bottomed watch-glasses. (4) A bulbous pipette. (5) Ordinary pipettes. (6) An india-rubber teat. (Plate IV. *a*.)

All aspiration in and out of pipettes is done by means of the teat. The practice of aspiration with the mouth is attended

with grave risk, and is entirely reprehensible. Experimentation with virulent bacteria is by no means without danger, and in view of fatal accidents which have occurred, there is no excuse for running this avoidable risk.

Method. A few drops of salt-solution are poured into a flat-bottomed watch-glass, a little of the culture is taken up from the agar with a platinum needle and transferred to the salt-solution. An even suspension of this is required, and it is made by sucking the suspension in and out of the bulbous pipette by means of the teat, until it is thoroughly mixed. The suspension is now sucked into a short pipette and drawn up into the stem above the capillary end, and the pipette is sealed off just below the point where it becomes conical. An emulsion so prepared is generally too strong for use; it is "centrifuged" for a few minutes in order to drive down all the larger masses of bacteria. The supernatant part is now withdrawn with a pipette and mixed by aspiration in and out of a fresh watch-glass: when mixed it is drawn up into the pipette, which is sealed up as before. There is now a suspension of bacteria in a miniature test-tube. The suspension required is a very weak one, and even after being "centrifuged" it may require to be diluted with still more salt-solution. Most suspensions should be slightly opalescent, but the emulsions of different kinds of bacteria are required of different strengths, and practical experience is necessary to select a suitable one (see note, *infra*). All the requirements for the estimation of the opsonic index are now ready. A trial experiment should be first made to test the strength of the emulsion.

The Opsonic Mixture.

A capillary pipette is taken, a spot is made about three-quarters of an inch from its fine extremity with a paraffin pencil, and the teat is fixed to its wide end.

It is required to take up equal volumes of corpuscles, emulsion and serum in that order into one tube.[1] These

[1] The order is important, if bacteria are taken first, there will be some contamination of the corpuscles, which will then be unsuitable for use with other bacteria, and if serum is taken first some will be introduced among the bacteria and the comparison of the different sera will be vitiated.

materials are most conveniently arranged by embedding the tubes containing the two former in a small dish of plasticine, or some other putty-like substance. The tubes are inclined at an angle of about 30°, pointing in the same direction, so that the contents can be withdrawn from each in rapid succession. The capsule containing the serum, the variable ingredient, generally lies on the bench and is taken in the hand when required. It is usual to put the plasticine opposite the worker's left hand, with the tubes pointing to the right. The whole of the capillary end of the blood-capsule is cut off so that the serum can be withdrawn. The pipette is taken up in the right hand by the teat, upon which light pressure is made; the lighter the pressure the more complete the control. The tip of the pipette, is put into the corpuscles, and the pressure on the teat relaxed until some of the corpuscles have been aspirated into the capillary tube as far as the mark. The pipette is withdrawn and the pressure on the teat slightly relaxed still further, so that the corpuscles are drawn a short way up the tube and an air-bubble enters it below them. The tip is wiped with the fingers of the left hand. The pipette is then put into the emulsion and an equal volume of this is drawn up. A further air-bubble is taken in and then a volume of serum. There are now three equal volumes in the pipette (Plate IV, fig. *a*). These are blown out by a gentle pressure on the teat on to a clean microscope slide. Very gentle pressure should be made until the serum forms a drop at the tip of the pipette, this is then lowered on to the slide, the pipette is raised and a similar drop is made of the emulsion which is next lowered into the drop of serum, and the corpuscles are also treated in the same way. In this way the blowing of air into the drop is avoided, and the mixture can be drawn up into the pipette in an unbroken column. It is aspirated in and out of the pipette about six times when it will be thoroughly mixed. It is then drawn up for the last time, allowed to flow up the stem till there is an air-bubble half-an-inch long between the mixture and the point of the pipette, and the point is sealed in the by-pass of the Bunsen burner (Plate IV, fig. *b*). The mixture of corpuscles, emulsion and serum, now requires to be incubated at 37° C. for a suitable time, generally fifteen minutes, which is an arbitrary

but convenient period.[1] The teat is removed from the pipette, and the pipette is placed in a tube of the opsonic incubator.

Preparation of the Opsonic Film. After incubation the white corpuscles have to be examined for the bacteria which they have ingested. For this purpose they are spread upon a microscope slide. The slides must be of good quality and smooth, they should be three inches long by an inch broad, and must be thoroughly rubbed with fine emery paper which assists even spreading of the film. Slides are not quite flat but are slightly convex on one side and concave on the other. If the convex side is underneath, the slide will rock on the microscope stage when pressure is put on one end, and the film will go out of focus. The convex side should therefore be uppermost, and the film should be spread upon it. The convex side may be determined by putting the slide on a flat surface and striking the edge near one extremity, if the convex side is undermost the slide will spin round.

The opsonic mixture is spread into a film in the following manner:—The pipette is withdrawn from the incubator and a teat fixed to the open end. This teat is perforated with a small hole near its open end, which can be covered with the finger when pressure is made upon it. The tip of the pipette, is broken off, and the mixture is blown on to a slide and sucked in and out of the pipette three or four times to mix it thoroughly. A drop of the opsonic mixture is now placed about half an inch from the end of the slide (Plate IV, fig. *c*). A second specially prepared slide is next taken, and inclined at an angle between 30° and 45° with the first slide. The end is placed in the drop of mixture which will now spread out along the edge of the second slide. The second slide is then drawn steadily along the length of the first slide, in order to pull the mixture along and so make a film ; more precise details are given below. Now this film

[1] Emulsions of Gram-negative organisms (*e.g.*, gonococci or coliform bacilli) should be thicker than those of Gram-positive organisms, because the former are digested by the leucocytes within a few minutes of phagocytosis, so that when the film is examined a number of "ghosts" are seen in the leucocytes, and uncertainty in counting is the result ; incubation is therefore continued for short periods of about eight instead of fifteen minutes, and the emulsion must be proportionately stronger to give the same average of bacteria per leucocyte.

is made with the view of examining the white corpuscles, and since these are the largest elements in the blood, they will be found at the end of the film where it was last touched by the spreading slide, since they will be dragged forward and the smaller elements—the red corpuscles—will be left behind. More than this, by the pressure exerted by the spreading slide, the leucocytes will be pressed out flat, so that their contained bacteria will be more readily visible than if they were allowed to retain their natural globular form. A matter of detail of some importance is the size of the drop of mixture with which the film is made. The film should end at a point somewhere near the middle of the slide. If the drop is too small, it will spread out too much and the corpuscles will be inconveniently far apart, being in fact too few, and vice versâ. A convenient drop is about 6 millimetres in diameter. Two films are made from each pipette, in case one is imperfectly spread, or suffers damage in staining.

Spreader. The spreading slide is prepared as follows (Plate III): A thin slide is selected, and the edge of it is rasped with the glass-cutting knife at one spot. The slide is now taken in the two hands, the thumbs are brought together, tip to tip, on the upper surface, and the middle phalanges of the index fingers are brought together underneath it opposite the point where the edge of the slide was scratched (Plate III, fig. *b*). The slide is snapped across by an upward movement of the wrists; it will break at the point rasped, and if the two halves are examined it will be found that one presents a concave and the other a convex edge (fig. *c*, *d*). It will be impossible to produce an edge without any curve. An edge is required which is as nearly straight as possible, and several slides must be broken across until such an edge is obtained. When this is done, the concave half is selected and the corners of this snapped off (fig. *e*). The spreader made of a half-slide may be inconveniently short, and if so it may be lengthened by fixing it to the end of another slide with sealing wax; for security it should overlap this slide by about half-an-inch (fig. *f*).

A spreader has now been made which has a concave edge, which is not quite as wide as the slide on which the film is to be made. The edge must be clean and sharp: sometimes one side of a spreader works better than the other, both

sides should be tried. When such a spreader is used the film obtained is oblong, and presents an upper, a lower and two lateral edges ; the lower edge is nearly straight, and in it the leucocytes will be collected. One object in spreading is to obtain as straight an edge as possible, since if it is much broken into a series of finger-like processes the specimen will not be satisfactory ; in the straight edge a long row of leucocytes is found which can be examined in rapid succession with great saving of time. In spreading a film, no great pressure need be made with the spreader. When the slide is inclined at the proper angle its own weight will be sufficient, only enough pressure being maintained to keep it in the line required.

The film should be made by a deliberate and steady movement of the hand, without snatching, but without delay, and should be carried on some distance down the slide after the film is finished, and no alteration in the angle of inclination of the spreader nor in the rate of movement should be made, when the lower edge is reached. The precise angle required varies with the degree of concavity of the spreader, the greater the concavity the smaller the angle, but the suitable angle for each spreader must be found by experiment.

The spreading of the film is of first rate importance and the following precise direction should be followed :

The object of spreading being to draw all the white corpuscles to the lower edge of the film, and to make this edge as straight as possible, it is important to make an even movement with the spreader, since if it is lifted at all some corpuscles will be left behind.

Hold the spreader by its lateral edges between the thumb and middle finger of the right hand about an inch from its prepared end, which points forward, the other end is directed towards the palm, and not between the thumb and index finger like a pen (Plate IV, fig. *d*). Place the tip of the index finger on its upper surface between the thumb and the middle finger (fig. *e*). If the hand and spreader are held in this position and are put on the bench so as to be in contact with it at three points, one of which is the edge of the spreader, the whole hand may be moved by retraction of the shoulder, and if the points of contact are maintained the spreader will move quite evenly (fig. *f*). The points of

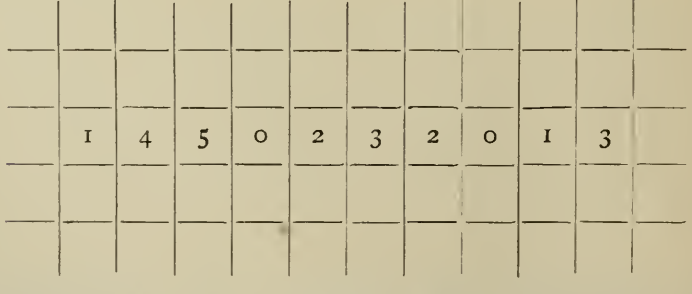
contact with the bench are: (1) In front: the edge of the spreader which moves along the slide, and must be in contact with it along the whole of its prepared edge. (2) At the side; the tips of the ring and little fingers. (3) Behind: the hypothenar eminence and pisiform bone. Since the whole hand is to move along the bench the latter should be perfectly smooth, as any inequalities on the surface will jerk the hand and the irregularities will be communicated to the film. A plate-glass slab makes the best surface, but if this is not to be had, a smooth wooden table should be used. To spread the film, suppose that you are facing the north, place the slide convex side upwards and well rubbed with fine emery paper, pointing north-west. Place a drop of mixture on the slide, half an inch from its north-west end, and midway between the sides. Take up the spreader as described, point it north-west and incline at an angle of about 30° with the slide, dip the prepared edge into the mixture keeping this parallel with the north-west edge of the slide and in contact with the slide in its whole width, neither corner being raised. Move the spreader laterally for a short distance right and left till the mixture runs out the whole width of the spreading edge. Hold the slide on the bench by the tips of the left thumb and index finger on its north-west corners, and only putting enough pressure on the spreader to keep it steady in position, draw its edge along the slide, maintaining the three points of contact as described. The movement is made deliberately, and at the rate at which one would follow out a line with a pencil if accurate tracing were required. As each slide is made it must be numbered with a paraffin pencil.

The film thus made may be fixed by any ordinary method, one of the most convenient methods is to place the slide film downwards over a wide-mouthed vessel containing 40 per cent. formalin. It is then stained with carbol-thiomine for three-quarters of a minute, washed in water, dried with blotting paper and examined. This first specimen is merely a trial to test whether the emulsion is of suitable strength.

Counting the Opsonic Index. The film is placed under the low power of the microscope, and the bottom edge where the leucocytes are collected is examined. The row of leucocytes can easily be seen with a 1 inch lens (Frontispiece, fig. *b*), and when this is arranged in the centre of the field

the 1/12th inch lens is put on. In a properly prepared film the row of leucocytes will be found so arranged that from 10 to 20 are to be seen in one field of the microscope, and part of the film can always be found where these conditions prevail (Frontispiece, fig. *c*).

It is required to find a place where the leucocytes are sufficiently close together to enable a large number to be counted in rapid succession, but not so close that there is a difficulty in distinguishing their outlines; in a part of the field in which a dozen leucocytes are touching one another their outlines cannot be made out, the nuclei are confused, and neither can the precise number of corpuscles in the mass be estimated nor the number of bacteria ingested by them. In a suitable part of the field the leucocytes will be found flattened out, and bacteria will be seen within their protoplasm. A suitable emulsion for most organisms is such that after a period of fifteen minutes' incubation the leucocytes contain an average of 4 bacteria.[1] If more than 6 are present in the leucocytes the counting becomes extremely wearisome and it is difficult to maintain accuracy. It will not be found that all the leucocytes contain bacteria in equal amount, many will contain none at all and some will contain considerably more than the average. It is usually considered enough to count 100 leucocytes and their contained bacteria. For beginners it is best to write down the numbers of bacteria in each leucocyte examined in a row or series of rows. Squared paper is convenient for this purpose: thus if the film shown in the illustration were to be counted the estimation would be made as follows, counting from the top:—



[1] The film shown in the frontispiece contains tubercle bacilli and was stained by the Ziehl-Neelsen method. Weaker emulsions are used for such specimens. (*Vide infra.*)

After some practice the number of leucocytes present in groups of 10 bacteria can be counted easily, and are usually put down in a horizontal row.

The specimen under consideration was put up as a trial to test the strength of the emulsion. If, using normal serum, a satisfactory count has been found, the estimation may be proceeded with at once. If the emulsion is too strong it may be diluted, or if not strong enough, either some stronger emulsion may be added or else the incubation may be carried on for a longer period. When it is desired to estimate the opsonic index of a patient's serum, the procedure is as follows :—

For staphylococcal infections the normal control is generally made by mixing equal quantities of serum from six healthy persons. It is not always possible to obtain such specimens, and in their absence it is best always to use the blood of the same healthy person as control, preferably one's own. If specimens from a large series of patients are to be examined, a convenient arrangement of the bench is as follows: The opsonic incubator stands at the back opposite the worker's seat within reach of the outstretched hand, but away from the materials. The corpuscles and emulsion stand opposite his left hand, near the edge of the bench, and, just to their right the capsules of serum lie in a vertical row, with their ends open and pointing to the right; their names or numbers should be written on the bench in the order in which they lie. A pile of rubbed slides lies within reach. The space immediately in front should be kept clear for mixing and spreading. Opposite the right hand is the Bunsen burner, with only the by-pass alight, and near by is a heap of pipettes, with their ends cut square and a fiduciary mark ready made on their capillary stems. The corpuscles and emulsion are as before allowed to stand in the plasticine, and each specimen of serum is taken up as required. A watch or clock with a second hand must be within sight, and the time at which each specimen is put into the incubator must be recorded, in order that all may be incubated for a similar period: thus, if the first is put in at five minutes past the hour, " 5 " is written down, and if the second takes a minute and a half to put up, " 6.30 " is put down; if the third takes one minute, " 7.30," and

so on. After fifteen minutes, *i.e.*, at twenty minutes past the hour, the first is withdrawn, the second at 21.30 and the third at 22.30, and so on. After some practice it is quite easy to put up each specimen in one minute, so that if the time when the first was put in is recorded, there is no need to record the others. The spreading and staining are proceeded with as in the trial experiment. In each specimen 100 leucocytes are counted with their contained bacteria. The leucocytes have been well mixed, and it is best to make a point of counting 100 consecutive leucocytes.

To estimate the opsonic index, the number of bacteria counted in 100 leucocytes in both the normal and abnormal specimens are separately added up. The results obtained with each patient's serum is divided by the result obtained with the normal. In this way the normal is regarded as unity, and each patient's index is expressed in terms of the normal, that is, as some decimal fraction or multiple of the normal. Thus if the total number of bacteria ingested by 100 leucocytes in the normal specimen is 310, and the number in two patients' specimen are 425 and 261 respectively, the patients' indices will be $\frac{425}{310}$ and $\frac{261}{310}$, or 1·37 and 0·71.

With regard to tubercle there are certain differences. An emulsion of tubercle is difficult to prepare. It is made from dried and sterilised tubercle bacilli which are first powdered in a glass mortar as fine as possible. The best pestle and mortar for the purpose are those devised by Captain A. F. Hayden, I.M.S., and manufactured by Mr. H. F. Angus. About ten "crumbs" of tubercle bacilli should be put into the mortar. A single drop of 1 per cent. saline solution is added and the bacilli are further ground up with this. Great pressure is not required, only a rotatory movement of the pestle, which should be maintained with the fingers. When the pestle begins to stick a few drops more of saline may be added and the grinding is kept up for about ten minutes. A few drops of salt are then poured in, stirred up, drawn off and reserved in a sedimentation tube. The grinding is resumed, more salt is added and more suspension drawn off and added to the

first: altogether 1 to 2 c.c. of solution is used to suspend the whole mass. The emulsion should now be "centrifuged" for about one minute, by which means the largest clumps are separated. The supernatant fluid is pipetted off and put into a second sedimentation tube. This is then placed in an empty test-tube, and the test-tube is immersed in water and boiled for ten minutes. This may be kept as a stock emulsion, and if preserved in an ice-chest it may be used for several weeks. The most suitable strength of a tubercle emulsion is one which gives an average of more than one and less than two bacteria per leucocyte. To find the suitable strength three or four samples of emulsion are taken and "centrifuged" for different periods such as 2, 4, 6 and 8 minutes and opsonic preparations are made with each. When a suitable strength is found it can be obtained from stock emulsion by "centrifuging" for the right period. For tuberculo-opsonic indices the normal is not estimated by a "pool" of healthy sera. It appears that all healthy persons have nearly similar indices to the tubercle bacillus, so that in all estimations two different normal sera are used, and the number of bacteria counted in 100 leucocytes in the two specimens should be within 10 per cent. of each other; the normal is considered to be the average of these two. When a large series of sera are examined, one in every ten should be a normal specimen.

Tubercle films are stained by the Ziehl-Neelsen method. The films are fixed by exposing to 40 per cent. formalin vapour for a few seconds. Carbol-fuchsin is boiled in a test-tube and is then immediately poured on to the specimen, and allowed to stand for five minutes. The stain is washed off with water and decolorised in 2·5 per cent. sulphuric acid for twenty seconds, and then thoroughly washed. Decolorisation is completed by pouring 4 per cent. acetic acid on to the specimen and washing this off immediately. This destroys the red corpuscles and accentuates the nuclei of the white. The acid must then be very thoroughly washed off because the counter-stain is made up of $\frac{1}{2}$ per cent. methylene blue and $\frac{1}{2}$ per cent. sodium carbonate, and if acid is present in the specimen the leucocytes may be damaged by the liberation of the carbonic acid. The specimen is stained with the methylene

blue for three-quarters of a minute, washed in water and dried rapidly, since the blue is easily washed out of the leucocytes. Otherwise the procedure for the tuberculo-opsonic index is the same for all others.

B. *The Preparation of Vaccines.* The method of making a staphylococcus vaccine will be described, which will serve as an example, all other vaccines are made on the same principle, with only trifling differences in detail.

The following materials are required. Agar in sloped test-tubes, 5 cubic centimetres of sterile sodium chloride solution (1 per cent.) in a sterile test-tube, plugged with sterile wool, a platinum needle, a blow-pipe, a vaccine sterilizer (*vide supra*) and the ordinary appliances available in a bacteriological laboratory.

A pure culture of staphylococcus is obtained, which is planted thickly on two or more agar tubes and incubated at 37° C. for twenty-four hours. By this time a thick growth will be obtained. The suspension is made as follows:— About five cubic centimetres of salt solution are poured into the first agar tube, and the colonies lightly rubbed off the agar with a platinum needle or a bent glass pipette with the end sealed, until the bacteria are suspended in the salt solution. The liquid is now poured into the other agar-tubes in succession and the process repeated in each case, after which it is poured back into a sterile test-tube which is closed with wool. This test-tube must be sealed in the blow-pipe flame, as described in the section on glass blowing.

There is now a suspension of staphylococci in salt solution, sealed up in a test-tube. The bacteria are, however, in thick clumps and masses, and not evenly distributed through the solution, and an even suspension is required. The tube is therefore shaken either by hand or machinery till the masses of bacteria are broken up and distributed evenly through the liquid; vigorous agitation must be kept up for about half an hour. The end of the tube is then rasped and broken off, and a few drops are withdrawn for standardization, the tube is closed up again by heating the open end in the flame, and the vaccine is sterilized by immersion in water which is kept at 60° C. for an hour. The tube may be simply tied to a

weight, or fixed in a wire cruet with a heavy foot made for the purpose. After sterilization the tube is temporarily opened again, and a further sample withdrawn, which is planted on agar, and incubated for twenty-four hours; if no growth occurs in this time, the sterility of the vaccine is assured.

The sample withdrawn for standardization is used as follows: It is required to calculate how many bacteria are present in unit volume. It is therefore mixed in definite proportions with a suspension of other bodies of known strength, the mixture is examined microscopically, and the numbers of bacteria and of the elements of the standard suspension which are present in a certain area are both counted. The numerical strength of the two suspensions is thus compared, and the strength of the bacterial suspension can be calculated.

Human blood contains five million red corpuscles per cubic millimetre and this is used as the standard of comparison. The finger is punctured, a drop of blood obtained, and a measured volume is aspirated into a capillary pipette,[1] after which four equal volumes of vaccine, separated by air bubbles are taken up. These are blown in and out of the pipette, and well mixed. A drop of the mixture is put on a microscope slide, and spread into a film which is fixed and stained and examined under the microscope. The blood corpuscles will be seen lying in the field, with the staphylococci amongst them. All the corpuscles and all the cocci are counted in a series of consecutive fields of the microscope, until 500 red corpuscles and the corresponding cocci have been counted. This is the area mentioned above. The counting is considerably simplified by cross-wires in the eye-piece of the microscope. If the top of the eye-piece is unscrewed, a ledge will be seen running round the inside of the barrel. A cardboard disc can be made to rest on this ledge. A round hole is cut out of the middle of the disc and across the hole four hairs, or fine glass rods are fixed with wax or gum as shown in the figure (Frontispiece, fig. *e*). The corpuscles and bacteria seen through the central square are more easily counted than those in the whole field, and a series of such squares are examined (Frontispiece, fig. *d*).

[1] For method of using pipettes see section on "Method" in "Technique of the Opsonic Index" *supra*.

The calculation of the number of cocci per unit volume is made as follows. Suppose 1625 cocci have been counted. The odd numbers are neglected and it is considered that 500 red corpuscles correspond to 1600 cocci. But four volumes of vaccine were taken to one volume of blood, therefore a volume of blood containing 500 red corpuscles will equal in size a volume of vaccine containing $\frac{1600}{4}$ or 400 cocci: and a volume of blood containing five million corpuscles will correspond to a volume of vaccine which contains four million cocci. But five million corpuscles are contained in one cubic millimetre of blood, therefore a cubic millimetre of vaccine contains four million cocci, and a cubic centimetre of vaccine contains four thousand million cocci.

There are therefore eventually about five cubic centimetres of duly sterilized vaccine containing 4,000 million cocci per cubic centimetre, or altogether about 20,000 million cocci. Such vaccine is of a greater strength than is suitable for inoculation purposes and it requires to be diluted. A convenient method of dilution is the following:—Bottles of suitable size and shape are sterilized and each charged with 25 cubic centimetres of sterile 1 per cent. salt-solution containing $\frac{1}{2}$ per cent. phenol. These are closed with tightly-fitting rubber caps and coated round the neck with melted paraffin to seal up any leak. Into these the vaccine is introduced in any desired strength. Suppose a strength of 500 million cocci per cubic centimetre is required. Twenty-five cubic centimetres of vaccine of this strength will require 12,500 million cocci, or $3\frac{1}{8}$ cubic centimetres of the undiluted vaccine. The rubber covered bottle is inverted, a sterilized hypodermic needle attached to a syringe is thrust through the cap and $3\frac{1}{8}$ cubic centimetres of salt solution are withdrawn and rejected. The test-tube containing the sterilized vaccine is now taken, and the end broken off. The tube is nverted, the needle of a sterile syringe is introduced and the required $3\frac{1}{8}$ cubic centimetres of vaccine is withdrawn (see Plate II, fig. *l*). This is discharged through the rubber cap into the vaccine bottle, which now contains 25 cubic centimetres of vaccine of the strength of 500 million staphylococci per cubic centimetre.

The only modifications are as follows, for the preparation

of vaccine on a large scale special bottles exposing large surfaces of medium are used instead of test-tubes. Some vaccines are very difficult to count, and with these the growth obtained from a certain area of medium is considered as the unit; a 25 cubic centimetre bottle may represent the amount obtained from the surface of agar exposed in one sloped test-tube and the dose is a certain fraction of that amount, obviously a far less accurate method of standardization.

Other vaccines may be sterilized by heating for shorter periods than an hour, at a slightly higher or lower temperature than 60° C. It is a good rule always to sterilize for the shortest possible period. The period of shaking required varies with different organisms, a vaccine of a motile organism such as the *bacillus coli* will break up in five minutes, a vaccine of some streptococci may require agitation for hours. Some diphtheroid organisms are very adherent; the vaccine prepared from them must be allowed to stand, the larger masses are allowed to settle and the supernatant fluid is pipetted off; this may contain sufficient organisms. These however are only differences of detail.

Tubercle vaccines are differently prepared. The best of these is the "bacillary emulsion" which is the one used in the cases quoted in this book. It is obtained from the makers (Meister, Lucius & Bruning) as a suspension of comminuted tubercle bacilli in glycerine and water, five milligrammes per cubic centimetre. It should be sterilized at 60° C, and diluted with salt solution till it contains 1/2,000 of a milligramme of comminuted bacilli per cubic centimetre. Lower strengths may be prepared as required. The tubercle vaccines are thus standardized by weight, not by numbers of organisms.

These are essential technical requirements for the practice of Therapeutic Inoculation.

PLATE I.

METHOD OF PREPARATION OF BLOOD CAPSULES
(actual size).

a A piece of glass tubing of required bore; heat at point I. *b* Tube drawn out after heating; fuse at II. *c* Same fused; reject right hand end and heat at III. *d* Same drawn out and while still hot bent into a curve as indicated by arrow; fuse at IV. *e* The capsule. *f* The same finished, curved end cut square, straight end drawn to a needle point.

Preparation of Welcker's pipette.—*g* Tubing of appropriate bore; heat at V. *h* The same drawn out; fuse at VI. *i* The same fused. *k* The same, end cut off square. *l* Bulbous pipette.

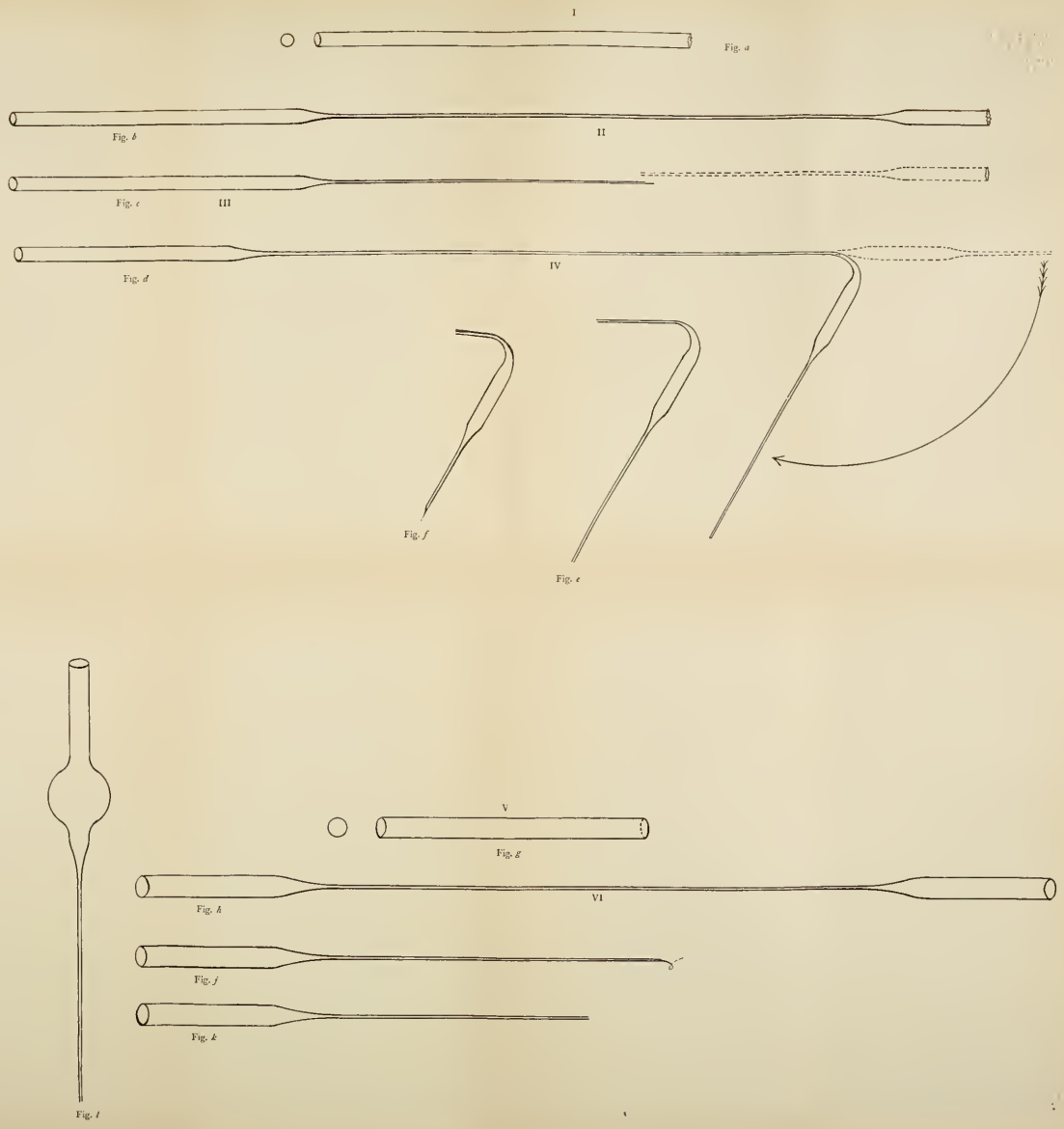


PLATE II.

METHOD OF PREPARING SEDIMENTATION TUBE
(actual size).

a Tubing with appropriate bore; heat at I. *b* The same drawn out; fuse at II. *c* The same fused; fuse again at III. *d* The same with roughly finished extremity; heat at IV and blow out extremity. *e* The same with rounded extremity and containing corpuscles.

Method of Sealing Test Tubes.—f Test tube containing vaccine; heat at V. *g* The same drawn out; fuse at VI. *h* The rough extremity cut off at VII; seal in small flame. *j* The same cut off. *k* Finished tube. *l* Method of withdrawing vaccine from capsule.

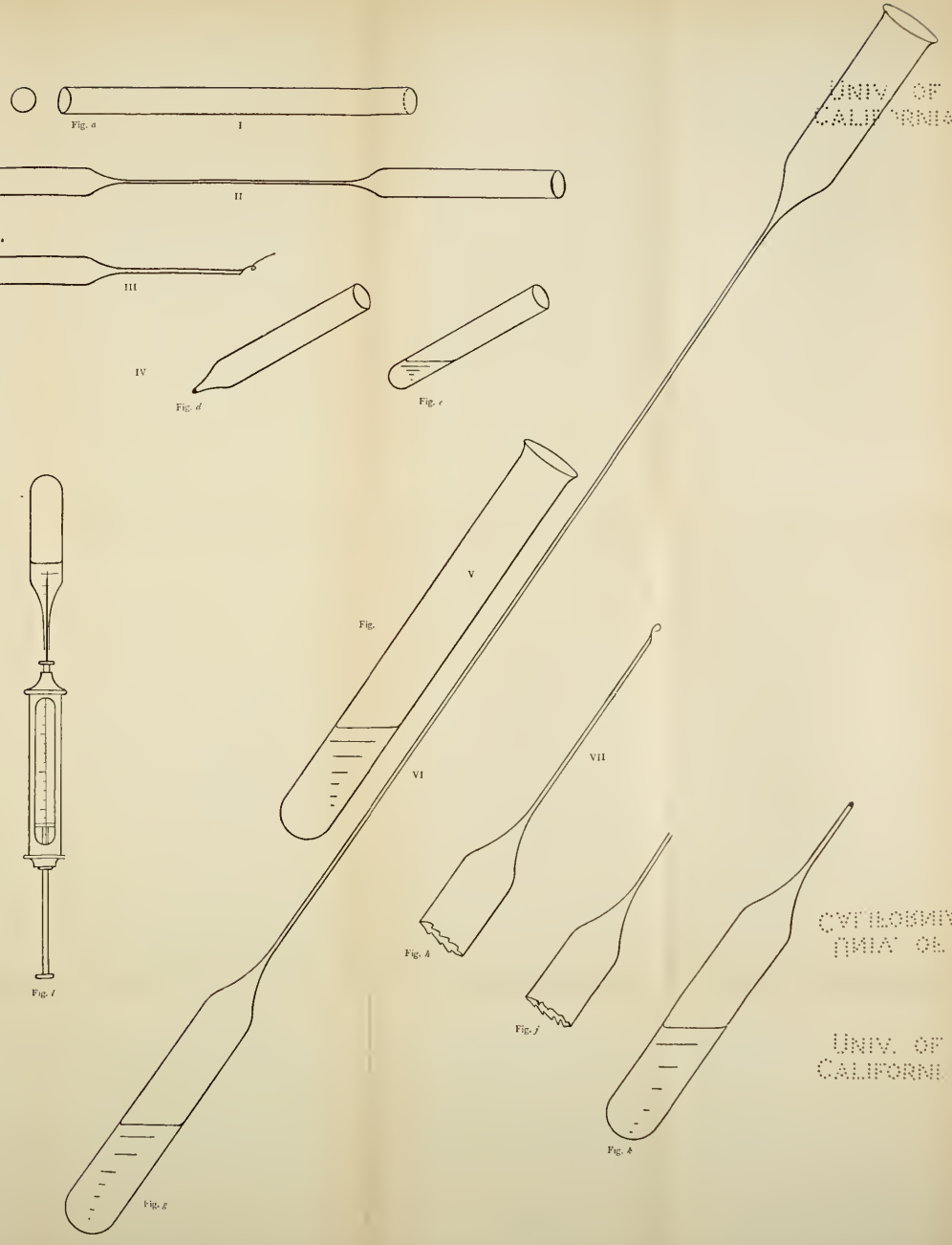


PLATE III.

PREPARATION OF SPREADER (actual size).

a Microscope slide rasped at I. *b* Hands in position for snapping slide. *c* Slide snapped across showing convex and concave halves. (The curve here represented is too marked.) *d* Shows a suitable curve. *e* The concave half with corners snapped off. *f* Finished spreader fixed to a second slide. *g* The spreader in position at correct angle for spreading film.

Fig. *a* I

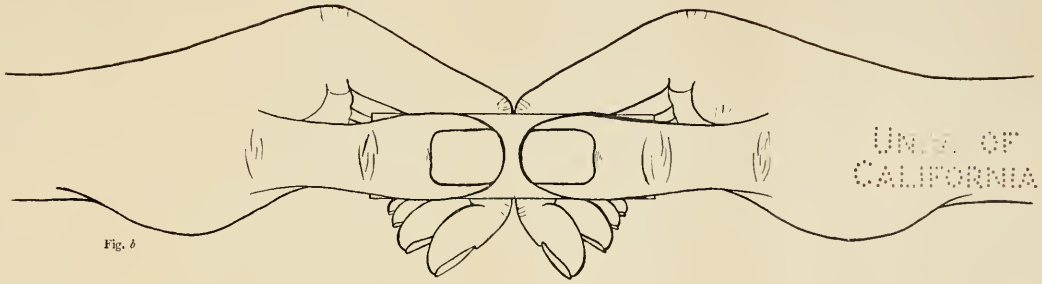


Fig. *b*

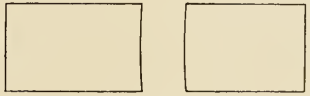


Fig. *c*

Fig. *d*

Fig. *e*

Fig. *f*

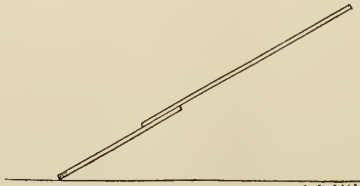


Fig. *g*

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PLATE IV

PREPARATION OF FILM FOR ESTIMATION OF THE OPSONIC INDEX (actual size).

a Pipette with perforated teat in position into which equal volumes of corpuscles, serum and emulsion have been aspirated. *b* The volumes mixed and drawn up in an even column without air bubbles. *c* A drop of mixture in position on slide. *d* First position of hands for holding the spreader. *e* Second position. *f* Third position, hands and spreader touching the bench or slide at three points. I Edge of the spreader (on slide). II Tips of ring and little fingers. III Hypothenar eminence and pisiform bone. All these points should be kept in contact with the bench or slide when spreading the film.

Fig. *a*

Fig. *b*

Fig. *c*

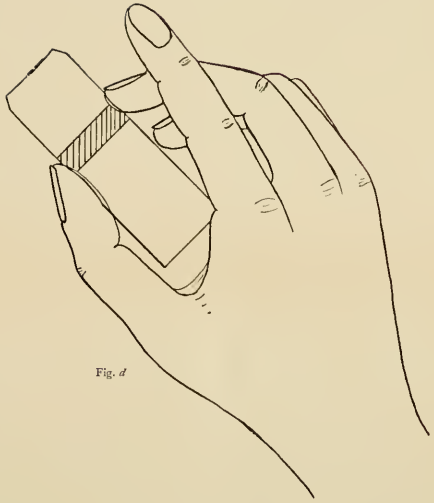

Fig. *d*

Fig. *e*

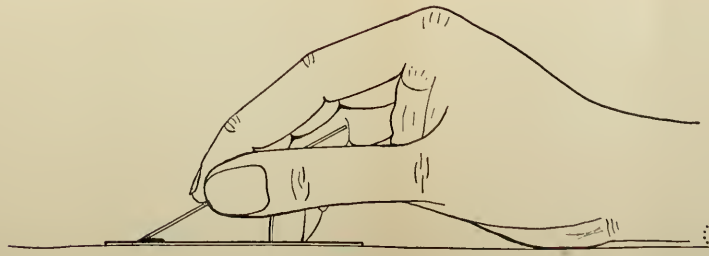

Fig. *f*

INDEX

INDEX

C

D

E

F

G

R. CLAY AND SONS, LTD., BREAD ST. HILL, E.C. AND BUNGAY, SUFFOLK